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[Continued on next page]

(54) Title: G-PROTEIN COUPLED RECEPTOR NUCLEIC ACIDS, POLYPEPTIDES, ANTIBODIES AND USES THEREOF

GGCGGCCCTT GCAAGGTTGC TGGACAGATG GAACTGGAAG GGCAGCCGTC  
TGCAGCCCAA GACACCCATT TCAAGCACTT TGAGTGACCA CGCGCTTGCAA  
GCTGGTGGCT GGCCCCCGGA CGCCGGGCT CTGAGGCACG GCGCTGACT  
TAAGCGTTGC ATCCTGTTAC CTGGAGACCC TCTGAGCTCT CACCTGCTAC  
TTCTGCGCT GCTTCTGCAC AGAGCCCGGG CGAGGACCCC TCCAGG  
ATGCAGGTC CGAACAGCAC CGGCCCGGAC AACGGACGC TGCAAGATGCT  
CGGGAAACCG GCGATCGGG TGGCCCTGCC CGTGGTGTAC TCGCTGGTGG  
CGGGCGGTGAG CATCCCGGGC AACCTCTCTCT CTCTGTGGGT GCTGTGCCGG  
CGCATGGGGC CCAGATCCCC GTCGGTACATC TTCAATGATCA ACCTGAGCGT  
CACGGACCTG ATGCTGGCCA GCGTGTGCC TTTCCAATAC TACTACCAATT  
GCAACCGCCA CCACCTGGTA TTCGGGGTGC TGCTTGCAA CGTGGTGC  
GTGGCCCTTTT ACACCAAACAT GTATTCCAGC ATTCCTACCA TGACCTGTAT  
CAGCGTGGAG CGCTTCCCTGG GGGTCTCGTA CCCGCTCAGC TCCAAGCGCT  
GGCGCGGCCG TCGTTACCGG GTGGCCCGGT GTGCAGGGAC CTGGCTGCTG  
CTCCTGACCC CCCGTGCCCC GCTGGCCGCC ACCGATCTCA CCTACCCGGT  
GCACGGCCCTG GGCATCATCA CCTGCTCGA CGTCTCAAG TGAGCGATG  
TCCCCCAGCTG GGCCTATGTCG CGCGTGTCTCC TCTTCACCAT CTCATCCCTG  
CTGTTCCCTCA TCCCCTGTCG GATCACCGTG GCTTGTACA CGGCCACCAT  
CCTCAAGCTG TTGCGCACCG AGGGAGGCAGA CGGGCGGGAG CAGCGGAGGC  
CGCGCGGTGG CGCTGGCCCG GTGGTCTCTG TGCCCTTGTG CACCTGCTTC  
GCCCGAACAA ACTTCGTCT CCTGGCGCAC ATCCGTGAGCC GCTCTTCTA  
CGGCAAGAGC TACTACCAAGC TGTAACAAGCT CACCGTGTGT CTCAGCTGCC  
TCAACAACTG TCTGGACCCG TTGTTTATT ACTTTGCGTC CGGGAAATT  
CAGCTGGCCG TCGGGGAAATA TTGGGGCTGC CGGGGGGTGC CGAGAGACAC  
CCTGGACACG CGCCGCGAGA GCCTCTCTC CGCCAGGACC ACGTCGGTGC  
GCTCCGAGGC CGGTGCCAC CCTGAAGGGG TGAGGGAGC CACCAAGGCC  
GGCCTCCAGA GGCAGGAGAG TGTGTTG  
TCCCTGCTGA CATCGTCCCT TAGTTGTTGCT TCTGGCCCTTC TCCATTCTCC  
TCCAGGGTT CTGGTCTCCG TAGCCCCGGTGC CACGCCGAAA TTTCTGTTA  
TTTCACTCAAG GGGCACTGTG GTGCTGTG TGGAATTCT TCTTTCAGAG  
GAGCGCCTGG GGCTCCCTGCA AGTCAGCTAC TCTCCGTGCC CACTTCCCT  
CACACACACA CCCCCCTCGT GCGAATTCT T

(57) Abstract: The invention provides isolated HGPRBMY1 and HGPRBMY2 nucleic acid molecules and polypeptide molecules. The invention also provides antisense nucleic acid molecules, expression vectors containing the nucleic acid molecules of the invention, host cells into which the expression vectors have been introduced, and non-human transgenic animals in which a nucleic acid molecule of the invention has been introduced or disrupted. The invention still further provides isolated polypeptides, fusion polypeptides, antigenic peptides and antibodies. Diagnostic, screening and therapeutic methods utilizing compositions of the invention are also provided.

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5 **G-PROTEIN COUPLED RECEPTOR NUCLEIC ACIDS,  
POLYPEPTIDES, ANTIBODIES AND USES THEREOF**

This application claims benefit to provisional application U.S. Serial No. 60/270,793, filed February 23, 2001; to provisional application U.S. Serial No. 60/270,792, filed February 23, 2001; and to provisional application U.S. Serial No. 60/296,427, filed June 6, 2001. The teachings of the referenced applications are incorporated herein by reference in their entirety.

## 1. INTRODUCTION

15 Many transmembrane proteins are receptors that bind a ligand and transduce an intracellular signal, leading to a variety of cellular responses. The identification and characterization of such a receptor enables one to identify both the ligands which bind to the receptor and the intracellular molecules and signal transduction pathways associated with the receptor, permitting one to identify or design modulators of receptor activity, e.g., receptor agonists or antagonists and modulators of signal transduction.

The present invention relates to the discovery and characterization of nucleic acid molecules that encode a G-protein coupled receptor (GPCR), a receptor that participates in signal transduction in eukaryotic cells. More specifically, the present invention relates 25 to a novel GPCR that is particularly expressed in bone marrow and spleen tissue, referred to herein as HGPRBMY1. The invention features GPCR nucleic acid molecules, host cell expression systems, GPCRs, fusion polypeptides, peptides, antibodies to the receptor, transgenic animals that express a GPCR transgene, or recombinant knock-out animals that do not express the GPCR, antagonists and agonists of the receptor, and other 30 compounds that modulate GPCR gene expression or GPCR activity that can be used for diagnosis, drug screening, clinical trial monitoring, and/or as pharmaceutical compositions the treatment of immune related diseases and disorders, particularly proliferative immune and autoimmune disorders, specifically p27 and/or IkB defects.

35 The present invention relates to the discovery and characterization of nucleic acid molecules that encode a G-protein coupled receptor (GPCR), a receptor that participates in signal transduction in eukaryotic cells. More specifically, the present invention relates

to a novel GPCR that is particularly expressed in heart and brain tissue, referred to herein  
5 as HGPRBMY2. The invention encompasses GPCR nucleic acid molecules, host cell  
expression systems, GPCR polypeptides, fusion polypeptides, peptides, antibodies to the  
receptor, transgenic animals that express a GPCR transgene, or recombinant knock-out  
10 animals that do not express the GPCR, antagonists and agonists of the receptor, and other  
compounds that modulate GPCR gene expression or GPCR activity that can be used for  
diagnosis, drug screening, clinical trial monitoring, and/or as pharmaceutical  
compositions the treatment of cardiovascular and/or neural diseases and disorders.

## 2. BACKGROUND OF THE INVENTION

15 G-protein coupled receptors (GPCRs) belong to one of the largest receptor  
superfamilies known. These receptors are biologically important and malfunction of these  
receptors results in diseases such as Alzheimer's, Parkinson, diabetes, dwarfism, color  
blindness, retinal pigmentosa and asthma. GPCRs are also important signaling molecules  
in subjects with depression, schizophrenia, sleeplessness, hypertension, anxiety, stress,  
20 renal failure and in several other cardiovascular, metabolic, neuro, oncology and immune  
disorders (Horn and Vriend, J. Mol. Med. 76:464-468, 1998). They have also been shown  
to play a role in HIV infection (Feng et al., (1996) Science 272:872-877).

25 GPCRs are integral membrane proteins characterized by the presence of seven  
hydrophobic transmembrane domains which span the plasma membrane and form a  
bundle of antiparallel alpha helices. The transmembrane domains account for structural  
and functional features of the receptor. In most cases, the bundle of helices forms a  
binding pocket; however, when the binding site must accommodate more bulky  
molecules, the extracellular N-terminal segment or one or more of the three extracellular  
30 loops participate in binding and in subsequent induction of conformational change in  
intracellular portions of the receptor. The activated receptor, in turn, interacts with an  
intracellular heterotrimeric G-protein complex which mediates further intracellular  
signaling activities, generally interaction with guanine nucleotide binding (G) proteins  
and the production of second messengers such as cyclic AMP (cAMP), phospholipase C,  
35 inositol triphosphate or ion channel proteins (Baldwin, J. M. (1994) Curr. Opin. Cell  
Biol. 6:180-190). The activity of the receptors are then modulated by modification, such  
as phosphorylation, or by binding to a regulatory molecule, such as by the negative

5 regulatory molecule arrestin, or by internalization wherein the receptor is degraded in a  
lyosome (see generally Hu, L.A., *et al.*, (2000) *J. Biol. Chem.* 275:38659-38666).

10 The amino-terminus of the GPCR is extracellular, of variable length and often  
glycosylated, while the carboxy-terminus is cytoplasmic. Extracellular loops of the GPCR  
alternate with intracellular loops and link the transmembrane domains. The most  
15 conserved domains of GPCRs are the transmembrane domains and the first two  
cytoplasmic loops. GPCRs range in size from under 400 to over 1000 amino acids  
(Coughlin, S. R. (1994) *Curr. Opin. Cell Biol.* 6:191-197).

15 GPCRs respond to a diverse array of ligands including lipid analogs, amino acids  
and their derivatives, peptides, cytokines, and specialized stimuli such as light, taste, and  
odor. GPCRs function in physiological processes including vision (the rhodopsins), smell  
(the olfactory receptors), neurotransmission (muscarinic acetylcholine, dopamine, and  
adrenergic receptors), and hormonal response (luteinizing hormone and  
thyroid-stimulating hormone receptors).

20 GPCR mutations, both of the loss-of-function and of the activating variety, have  
been associated with numerous human diseases (Coughlin, *supra*). For instance, retinitis  
pigmentosa may arise from either loss-of-function or activating mutations in the  
rhodopsin gene. Somatic activating mutations in the thyrotropin receptor cause  
25 hyperfunctioning thyroid adenomas (Parma, J. *et al.* (1993) *Nature* 365:649-651). Parma  
*et al.* suggest that certain G-protein-coupled receptors susceptible to constitutive  
activation may behave as proto-oncogenes.

25 Characterization of the HGPRBMY1 polypeptide of the present invention led to  
the determination that it is involved in the modulation of the cyclin p27 protein, in  
addition to, the apoptosis regulatory protein I $\kappa$ B, either directly or indirectly. The present  
30 invention represents the first association between HGPRBMY1 to cell cycle and  
apoptosis regulation.

35 Critical transitions through the cell cycle are highly regulated by distinct protein  
kinase complexes, each composed of a cyclin regulatory and a cyclin-dependent kinase  
(cdk) catalytic subunit (for review see Draetta, 1994). These proteins regulate the cell's  
progression through the stages of the cell cycle and are in turn regulated by numerous  
proteins, including p53, p21, p16, p27, and cdc25. Downstream targets of cyclin-cdk  
complexes include pRb and E2F. The cell cycle often is dysregulated in neoplasia due to

alterations either in oncogenes that indirectly affect the cell cycle or in tumor suppressor genes or oncogenes that directly impact cell cycle regulation, such as pRb, p53, p16, cyclin D1, or mdm-2 (for review see Lee and Yang, 2001, Schafer, 1998).

10 P27, also known as CDNK1B (cyclin-dependent kinase inhibitor 1B) or KIP1, shares a limited similarity with the CDK inhibitor CDKN1A/p21. The encoded protein binds to and prevents the activation of cyclinE-CDK2 or cyclinD-CDK4 complexes. Therefore it mainly blocks the cell cycle progression at the G1- and S-phases (for review see Desdouets and Brechot, 2000).

15 Reduction in levels of p27 and increased expression of cyclin E also occur and carry a poor prognostic significance in many common forms of cancer. The inhibition of protein activities leading to an upregulation of p27 might therefore be a possibility to decrease the progression of cancer and increase patient survival rates (for review see Sgambato, 2000).

20 Recently, Medema et al. (2000) demonstrated that p27 is a major transcriptional target of forkhead transcription factors FKHRL1, AFX, or FKHR. Overexpression of these proteins causes growth suppression in a variety of cell lines, including a Ras-transformed cell line and a cell line lacking the tumor suppressor PTEN integrating signals from PI3K/PKB signaling and RAS/RAL signaling to regulate transcription of p27(KIP1). Expression of AFX blocked cell cycle progression at phase G1, independent 25 of functional retinoblastoma protein but dependent on the cell cycle inhibitor p27 (KIP1). This is further supported by the fact that AFX activity inhibits p27  $-/-$  knockout mouse cells significantly less than their p27  $+/+$  counterparts.

30 The connection between the PTEN pathway and the activation of p27 via forkhead-like transcription factors implies that genes whose inhibition leads to p27 upregulation might be involved in this pathway. Therefore the identification of genes 35 whose knockout leads to an upregulation of p27 might be useful drug targets, as inhibition of such genes should result in the upregulation of p27 and therefore be useful for the treatment and/or amelioration of cancer and increase a cancer patients prolonged outlook and survival.

35 The fate of a cell in multicellular organisms often requires choosing between life and death. This process of cell suicide, known as programmed cell death or apoptosis, occurs during a number of events in an organisms life cycle, such as for example, in

development of an embryo, during the course of an immunological response, or in the  
5 demise of cancerous cells after drug treatment, among others. The final outcome of cell  
survival versus apoptosis is dependent on the balance of two counteracting events, the  
onset and speed of caspase cascade activation (essentially a protease chain reaction), and  
the delivery of antiapoptotic factors which block the caspase activity (Aggarwal B.B.  
10 Biochem. Pharmacol. 60, 1033-1039, (2000); Thornberry, N. A. and Lazebnik, Y.  
Science 281, 1312-1316, (1998)).

The production of antiapoptotic proteins is controlled by the transcriptional factor  
complex NF- $\kappa$ B. For example, exposure of cells to the protein tumor necrosis factor  
(TNF) can signal both cell death and survival, an event playing a major role in the  
15 regulation of immunological and inflammatory responses (Ghosh, S., May, M. J., Kopp,  
E. B. Annu. Rev. Immunol. 16, 225-260, (1998); Silverman, N. and Maniatis, T., Genes  
& Dev. 15, 2321-2342, (2001); Baud, V. and Karin, M., Trends Cell Biol. 11, 372-377,  
(2001)). The anti-apoptotic activity of NF- $\kappa$ B is also crucial to oncogenesis and to  
chemo- and radio-resistance in cancer (Baldwin, A.S., J. Clin. Inves. 107, 241-246,  
20 (2001)).

Nuclear Factor- $\kappa$ B (NF- $\kappa$ B), is composed of dimeric complexes of p50 (NF- $\kappa$ B1)  
or p52 (NF- $\kappa$ B2) usually associated with members of the Rel family (p65, c-Rel, Rel B)  
which have potent transactivation domains. Different combinations of NF- $\kappa$ B/Rel  
25 proteins bind distinct kB sites to regulate the transcription of different genes. Early work  
involving NF- $\kappa$ B suggested its expression was limited to specific cell types, particularly  
in stimulating the transcription of genes encoding kappa immunoglobulins in B  
lymphocytes. However, it has been discovered that NF- $\kappa$ B is, in fact, present and  
inducible in many, if not all, cell types and that it acts as an intracellular messenger  
30 capable of playing a broad role in gene regulation as a mediator of inducible signal  
transduction. Specifically, it has been demonstrated that NF- $\kappa$ B plays a central role in  
regulation of intercellular signals in many cell types. For example, NF- $\kappa$ B has been  
shown to positively regulate the human beta-interferon (beta-IFN) gene in many, if not  
35 all, cell types. Moreover, NF- $\kappa$ B has also been shown to serve the important function of  
acting as an intracellular transducer of external influences.

The transcription factor NF- $\kappa$ B is sequestered in an inactive form in the cytoplasm  
as a complex with its inhibitor, I $\kappa$ B, the most prominent member of this class being I $\kappa$ B $\alpha$ .

A number of factors are known to serve the role of stimulators of NF- $\kappa$ B activity, such 5 as, for example, TNF. After TNF exposure, the inhibitor is phosphorylated and proteolytically removed, releasing NF- $\kappa$ B into the nucleus and allowing its transcriptional activity. Numerous genes are upregulated by this transcription factor, among them I $\kappa$ B $\alpha$ . The newly synthesized I $\kappa$ B $\alpha$  protein inhibits NF- $\kappa$ B, effectively shutting down further transcriptional activation of its downstream effectors. However, as mentioned above, the 10 I $\kappa$ B $\alpha$  protein may only inhibit NF- $\kappa$ B in the absence of I $\kappa$ B $\alpha$  stimuli, such as TNF stimulation, for example. Other agents that are known to stimulate NF- $\kappa$ B release, and thus NF- $\kappa$ B activity, are bacterial lipopolysaccharide, extracellular polypeptides, chemical agents, such as phorbol esters, which stimulate intracellular phosphokinases, 15 inflammatory cytokines, IL-1, oxidative and fluid mechanical stresses, and Ionizing Radiation (Basu, S., Rosenzweig, K. R., Youmell, M., Price, B. D, Biochem, Biophys, Res, Commun., 247(1):79-83, (1998)). Therefore, as a general rule, the stronger the 20 insulting stimulus, the stronger the resulting NF- $\kappa$ B activation, and the higher the level of I $\kappa$ B $\alpha$  transcription. As a consequence, measuring the level of I $\kappa$ B $\alpha$  RNA can be used as a marker for antiapoptotic events, and indirectly, for the onset and strength of pro-apoptotic events.

The upregulation of I $\kappa$ B $\alpha$  due to the downregulation of HGPRBMY1 places this 25 GPCR protein into a signalling pathway potentially involved in apoptotic events. This gives the opportunity to regulate downstream events via the activity of the protein HGPRBMY1 with antisense polynucleotides, polypeptides or low molecular chemicals with the potential of achieving a therapeutic effect in cancer, and autoimmune diseases. In addition to cancer and immunological disorders, NF- $\kappa$ B has significant roles in other 30 diseases (Baldwin, A. S., J. Clin Invest. 107, :3-6 (2001)). NF- $\kappa$ B is a key factor in the pathophysiology of ischemia-reperfusion injury and heart failure (Valen, G., Yan, ZQ, Hansson, GK, J. Am. Coll. Cardiol. 38, 307-14 (2001)). Furthermore, NF- $\kappa$ B has been found to be activated in experimental renal disease (Guirarro C, Egido J., Kidney Int. 59, 415-425 (2001)). As HGPRBMY1 is highly expressed in bone marrow and spleen and there is the potential of an involvement in immune diseases.

35 The discovery of a new human G-protein coupled receptor as described herein, and the nucleic acids encoding it satisfies a need in the art by providing new compositions which are useful in the diagnosis, prevention and treatment of immune

disorders, and particularly those G-protein coupled receptors that modulate the p27  
5 and/or NFkB pathways.

### 3. SUMMARY OF THE INVENTION

HGPRBMY1 is a putative G-protein coupled receptor (GPCR) that is expressed  
10 in tissues, in particular immune system tissues such as bone marrow, spleen and thymus.  
10 More specifically, HGPRBMY1 comprises the amino acid sequences depicted in Figure  
2 which is encoded by the nucleic acid sequence depicted in Figure 1.

HGPRBMY2 is predicted to be a G-protein coupled receptor (GPCR) that is  
expressed in heart and brain tissue. More specifically, HGPRBMY2 comprises the amino  
15 acid sequences depicted in Figure 7 which is encoded by the nucleic acid sequence  
depicted in Figure 6. The clone encoding the HGPRBMY2 polypeptide was deposited  
with the ATCC as ATCC Deposit Number XXXXX on XXXXX.

As HGPRBMY1 and HGPRBMY2 have homology to GPCRs, they are likely  
20 seven transmembrane proteins located at the membrane of a cell. Signal transduction  
from GPCRs is triggered by the binding of agonists or antagonists to the receptor.  
Secondary regulation of the receptor may occur through post-stimulatory modification  
of the polypeptide (e.g., phosphorylation) and/or by binding to a secondary regulatory  
molecule, particularly on a cytoplasmic domain of the receptor (e.g., arrestin).

HGPRBMY1 mRNA has been detected in the bone marrow, spleen and thymus.  
25 Thus, neutralization of HGPRBMY1 agonists or antagonists, removal of HGPRBMY1  
agonists or antagonists, or interference with binding to HGPRBMY1 may result in  
improvement or prevention of immune related disease.

HGPRBMY2 mRNA has been detected in the heart, and various tissues of the  
30 brain. Thus, neutralization of HGPRBMY2 agonists or antagonists, removal of  
HGPRBMY2 agonists or antagonists, or interference with binding to HGPRBMY2 may  
result in improvement or prevention of cardiovascular and/or neurological diseases.

The invention features the use of HGPRBMY1 nucleic acid molecules,  
35 HGPRBMY1 polypeptides and peptides, fusion polypeptides or fusion peptides (e.g.,  
fusions to heterologous sequences), as well as antibodies to the HGPRBMY1 (which can,  
for example, act as HGPRBMY1 agonists or antagonists), antagonists that inhibit  
receptor activity or expression, or agonists that activate receptor activity or increase its

expression in the diagnosis and treatment of immune system or immune response  
5 diseases and/or disorders including, but not limited to immune system diseases or disorders in animals, including humans, particularly proliferative immune disorders, and autoimmune disorders.

The invention features the use of HGPRBMY2 nucleic acid molecules, HGPRBMY2 polypeptides and peptides, fusion polypeptides or fusion peptides (e.g.,  
10 fusions to heterologous sequences), as well as antibodies to the HGPRBMY2 (which can, for example, act as HGPRBMY2 agonists or antagonists), antagonists that inhibit receptor activity or expression, or agonists that activate receptor activity or increase its expression in the diagnosis and treatment of the cardiovascular system diseases or  
15 disorders, in addition to neural disorders, in animals, including humans.

The diagnosis of an HGPRBMY1 abnormality in a patient, or an abnormality in the HGPRBMY1 signal transduction pathway, will assist in devising a proper treatment or therapeutic regimen for immune disorders. In addition, HGPRBMY1 nucleic acid molecules and HGPRBMY1 polypeptides are useful for the identification of compounds  
20 effective in the treatment of immune disorders regulated by the HGPRBMY1, particularly proliferative immune disorders, and autoimmune disorders.

The diagnosis of an HGPRBMY2 abnormality in a patient, or an abnormality in the HGPRBMY2 signal transduction pathway, will assist in devising a proper treatment or therapeutic regimen for heart failure. In addition, HGPRBMY2 nucleic acid molecules and HGPRBMY2 polypeptides are useful for the identification of compounds effective  
25 in the treatment of cardiovascular and/or neural disorders regulated by the HGPRBMY2.

In particular, the invention described in the subsections below features HGPRBMY1, polypeptides or peptides corresponding to functional domains of the  
30 HGPRBMY1 (e.g., extracellular domain (ECD), transmembrane domain (TM) or cytoplasmic domain (CD)), mutated, truncated or deleted HGPRBMY1 (e.g., an HGPRBMY1 with one or more functional domains or portions thereof deleted, such as  $\Delta$ TM and/or  $\Delta$ CD), HGPRBMY1 fusion polypeptides (e.g., an HGPRBMY1 or a functional domain of HGPRBMY1, such as the ECD, fused to an unrelated polypeptide  
35 or peptide such as an immunoglobulin constant region, i.e., Ig-Fc), nucleic acid sequences encoding such products, and host cell expression systems that can produce such HGPRBMY1 products.

The invention also features antibodies and anti-idiotypic antibodies (including 5 Fab fragments), antagonists and agonists of the HGPRBMY1, as well as compounds or nucleic acid constructs that inhibit expression of the HGPRBMY1 gene (transcription factor inhibitors, antisense and ribozyme molecules, or gene or regulatory sequence replacement constructs), or promote expression of HGPRBMY1 (e.g., expression constructs in which HGPRBMY1 coding sequences are operatively associated with 10 expression control elements such as promoters, promoter/enhancers, etc.). The invention also relates to host cells and animals genetically engineered to express the human HGPRBMY1 (or mutants thereof) or to inhibit or "knock-out" expression of the animal's endogenous HGPRBMY1.

15 The HGPRBMY1 polypeptides or peptides, HGPRBMY1 fusion polypeptides, HGPRBMY1 nucleic acid sequences, antibodies, antagonists and agonists can be useful for the detection of mutant HGPRBMY1 or inappropriately expressed HGPRBMY1 for the diagnosis of immune disorders. The HGPRBMY1 polypeptides or peptides, HGPRBMY1 fusion polypeptides, HGPRBMY1 nucleic acid sequences, host cell 20 expression systems, antibodies, antagonists, agonists and genetically engineered cells and animals can be used for screening for drugs effective in the treatment of such immune disorders. The use of engineered host cells and/or animals may offer an advantage in that such systems allow not only for the identification of compounds that bind to the ECD of 25 the HGPRBMY1, but can also identify compounds that affect the signal transduced by the activated HGPRBMY1.

The HGPRBMY2 polypeptides or peptides, HGPRBMY2 fusion polypeptides, HGPRBMY2 nucleic acid sequences, antibodies, antagonists and agonists can be useful for the detection of mutant HGPRBMY2 or inappropriately expressed HGPRBMY2 for 30 the diagnosis of heart disease or neural disorders. The HGPRBMY2 polypeptides or peptides, HGPRBMY2 fusion polypeptides, HGPRBMY2 nucleic acid sequences, host cell expression systems, antibodies, antagonists, agonists and genetically engineered cells and animals can be used for screening for drugs effective in the treatment of such heart 35 disease or immune disorders. The use of engineered host cells and/or animals may offer an advantage in that such systems allow not only for the identification of compounds that bind to the ECD of the HGPRBMY2, but can also identify compounds that affect the signal transduced by the activated HGPRBMY2.

The HGPRBMY1 polypeptide products (especially soluble derivatives such as peptides corresponding to the HGPRBMY1 ECD, or soluble polypeptides lacking one or more TM domains ("ΔTM")), fusion polypeptides (especially HGPRBMY1-Ig fusion polypeptides, *i.e.*, fusions of the HGPRBMY1 or a domain of the HGPRBMY1, *e.g.*, ECD, ΔTM or CD to a heterologous sequence such as IgFc), antibodies and anti-idiotypic antibodies (including Fab fragments), antagonists or agonists (including compounds that modulate signal transduction which may act on downstream targets in the HGPRBMY1 signal transduction pathway) can be used for therapy of such diseases. For example, the administration of an effective amount of a pharmaceutical composition comprising soluble HGPRBMY1 ECD, ΔTM HGPRBMY1 or an ECD-IgFc fusion polypeptide or an anti-idiotypic antibody (or its Fab) that mimics the HGPRBMY1 ECD would modulate endogenous HGPRBMY1 agonists or antagonists, and prevent or reduce binding and receptor activation, leading to prevention of immune disorders.

For example, the administration of an effective amount of a pharmaceutical composition comprising a fusion polypeptide or an anti-idiotypic antibody, or fragment thereof, that mimics HGPRBMY1 would modulate endogenous HGPRBMY1 binding to signaling partners, leading to treatment of immune disorders, particularly proliferative immune disorders, and autoimmune disorders.

For example, the administration of an effective amount of a pharmaceutical composition comprising soluble HGPRBMY2 ECD, ΔTM HGPRBMY2 or an ECD-IgFc fusion polypeptide or an anti-idiotypic antibody (or its Fab) that mimics the HGPRBMY2 ECD would modulate endogenous HGPRBMY2 agonists or antagonists, and prevent or reduce binding and receptor activation, leading to prevention of heart failure.

Nucleic acid constructs encoding such HGPRBMY1 products can be used to genetically engineer host cells to express such HGPRBMY1 products *in vivo*; these genetically engineered cells, when placed in the body, deliver a continuous supply of HGPRBMY1 polypeptides or peptides, that modulate HGPRBMY1 activity. Nucleic acid constructs encoding functional HGPRBMY1, mutant HGPRBMY1, or antisense and ribozyme molecules can be used in gene therapy approaches for the modulation of HGPRBMY1 activity in the treatment of immune disorders, particularly proliferative immune disorders, and autoimmune disorders.

Nucleic acid constructs encoding such HGPRBMY2 products can be used to  
5 genetically engineer host cells to express such HGPRBMY2 products *in vivo*; these  
genetically engineered cells deliver a continuous supply of soluble HGPRBMY2 peptide,  
ECD or  $\Delta$ TM or HGPRBMY2 fusion polypeptide that will modulate activation of  
HGPRBMY2 by agonists or antagonists. Nucleic acid constructs encoding functional  
10 HGPRBMY2, mutant HGPRBMY2, as well as antisense and ribozyme molecules can be  
used in "gene therapy" approaches for the modulation of HGPRBMY2 expression and/or  
activity in the treatment of heart disease or neural disorders.

The invention also features HGPRBMY1 pharmaceutical formulations and  
methods for treating immune disorders, particularly proliferative immune disorders, and  
15 autoimmune disorders.

Thus, the invention also encompasses HGPRBMY2 pharmaceutical formulations  
and methods for treating heart or neural diseases.

The invention further relates to a method of identifying a compound that  
modulates the biological activity of HGPRBMY1 or HGPRBMY2, comprising the steps  
20 of, (a) combining a candidate modulator compound with HGPRBMY1 or HGPRBMY2  
having the sequence set forth in one or more of SEQ ID NO:2; and measuring an effect  
of the candidate modulator compound on the activity of HGPRBMY1 or HGPRBMY2.

The invention further relates to a method of identifying a compound that  
modulates the biological activity of a GPCR, comprising the steps of, (a) combining a  
25 candidate modulator compound with a host cell expressing HGPRBMY1 or  
HGPRBMY2 having the sequence as set forth in SEQ ID NO:2; and, (b) measuring an  
effect of the candidate modulator compound on the activity of the expressed  
HGPRBMY1 or HGPRBMY2.

30 The invention further relates to a method of identifying a compound that  
modulates the biological activity of HGPRBMY1 or HGPRBMY2, comprising the steps  
of, (a) combining a candidate modulator compound with a host cell containing a vector  
described herein, wherein HGPRBMY1 or HGPRBMY2 is expressed by the cell; and,  
35 (b) measuring an effect of the candidate modulator compound on the activity of the  
expressed HGPRBMY1 or HGPRBMY2.

The invention further relates to a method of screening for a compound that is

capable of modulating the biological activity of HGPRBMY1 or HGPRBMY2,  
5 comprising the steps of: (a) providing a host cell described herein; (b) determining the  
biological activity of HGPRBMY1 or HGPRBMY2 in the absence of a modulator  
compound; (c) contacting the cell with the modulator compound; and (d) determining  
the biological activity of HGPRBMY1 or HGPRBMY2 in the presence of the modulator  
10 compound; wherein a difference between the activity of HGPRBMY1 or HGPRBMY2  
in the presence of the modulator compound and in the absence of the modulator  
compound indicates a modulating effect of the compound.

The invention further relates to a recombinant host cell comprising a vector  
comprising all or a portion of the polynucleotide of SEQ ID NO:1 or SEQ ID NO:13 ,  
15 NFAT/CRE, and/or NFAT G alpha 15 wherein said host cell exhibits low levels of  
HGPRBMY1 or HGPRBMY2 expression. Such host cells are particularly useful in  
methods of screening for agonists of the HGPRBMY1 or HGPRBMY2 polypeptide.

The invention further relates to a recombinant host cell comprising a vector  
comprising all or a portion of the polynucleotide of SEQ ID NO:1 or SEQ ID NO:13 ,  
20 NFAT/CRE, and/or NFAT G alpha 15 wherein said host cell exhibits intermediate levels  
of HGPRBMY1 or HGPRBMY2 expression. Such host cells are particularly useful in  
methods of screening for modulators of the HGPRBMY1 or HGPRBMY2 polypeptide.

The invention further relates to a recombinant host cell comprising a vector  
25 comprising all or a portion of the polynucleotide of SEQ ID NO:1 or SEQ ID NO:13 ,  
NFAT/CRE, and/or NFAT G alpha 15 wherein said host cell exhibits high levels of  
HGPRBMY1 or HGPRBMY2 expression. Such host cells are particularly useful in  
methods of screening for antagonists of the HGPRBMY1 or HGPRBMY2 polypeptide.

The invention further relates to a method of screening for candidate compounds  
30 capable of modulating activity of a G-protein coupled receptor-encoding polypeptide,  
comprising the steps of contacting a test compound with a cell or tissue expressing all or  
a portion of the polynucleotide of SEQ ID NO:1 or SEQ ID NO:13 , NFAT/CRE, and/or  
NFAT G alpha 15 wherein said cell or tissue exhibits low, intermediate, or high  
35 HGPRBMY1 or HGPRBMY2 expression levels, and selecting as candidate modulating  
compounds those test compounds that modulate activity of the the HGPRBMY1 or  
HGPRBMY2 polypeptide.

5 The invention relates to a method of preventing, treating, or ameliorating a medical condition, comprising administering to a mammalian subject a therapeutically effective amount of the polypeptide of SEQ ID NO:2 or the polynucleotide of SEQ ID NO:1, wherein the medical condition is a proliferative disorder.

10 More preferably, the invention relates to a method of preventing, treating, or ameliorating a medical condition, comprising administering to a mammalian subject a therapeutically effective amount of an antagonist of the polypeptide of SEQ ID NO:2 or the polynucleotide of SEQ ID NO:1, wherein the medical condition is a proliferative disorder.

15 More preferably, the invention relates to a method of preventing, treating, or ameliorating a medical condition, comprising administering to a mammalian subject a therapeutically effective amount of an antagonist of the polypeptide of SEQ ID NO:2 or the polynucleotide of SEQ ID NO:1, wherein the medical condition is a disorder related to aberrant apoptosis regulation.

20 Alternatively, the invention relates to a method of preventing, treating, or ameliorating a medical condition, comprising administering to a mammalian subject a therapeutically effective amount of an agonist of the polypeptide of SEQ ID NO:2 or the polynucleotide of SEQ ID NO:1, wherein the medical condition is a proliferative disorder.

25 More preferably, the invention relates to a method of preventing, treating, or ameliorating a medical condition, comprising administering to a mammalian subject a therapeutically effective amount of an agonist of the polypeptide of SEQ ID NO:2 or the polynucleotide of SEQ ID NO:1, wherein the medical condition is a disorder related to aberrant apoptosis regulation.

30 The invention further relates to peptides that bind to the HGPRBMY1 or HGPRBMY2 polypeptide. More preferred are peptides that modulate the activity of HGPRBMY1 or HGPRBMY2 activity.

35 The invention further relates to a method for identifying compounds that regulate immune-related disorders, comprising the step of contacting a test compound with a cell which expresses a nucleic acid of SEQ ID NO:1, and determining whether the test compound modulates HGPRBMY1 activity.

The invention further relates to a method for identifying compounds  
5 that regulate immune-related disorders comprising the step of contacting a test  
compound with a nucleic acid of SEQ ID NO:1; and determining whether the test  
compound interacts with the nucleic acid of SEQ ID NO:1.

The invention further relates to a method for identifying compounds  
that regulate immune-related disorders, comprising the step of contacting a test  
10 compound with a cell or cell lysate containing a reporter gene operatively associated  
with a HGPRBMY1 regulatory element; and detecting expression of the reporter gene  
product.

The invention further relates to a method for identifying compounds  
that regulate immune-related disorders comprising the step of contacting a test  
15 compound with a cell or cell lysate containing HGPRBMY1 transcripts; and detecting  
the translation of the HGPRBMY1 transcript.

The invention further relates to a method for modulating  
immune-related disorders in a subject, comprising administering to the subject a  
therapeutically effective amount of a HGPRBMY1 polypeptide.

20 The invention further relates to a method for modulating immune-related  
disorders in a subject, comprising administering to the subject a therapeutically  
effective amount of a HGPRBMY1 polypeptide wherein the HGPRBMY1  
polypeptide is HGPRBMY1 or a functionally equivalent derivative thereof, preferably  
wherein the subject is a human.

25 The invention further relates to a method for modulating immune-related  
disorders in a subject, comprising administering to the subject a therapeutically  
effective amount of a HGPRBMY1 polypeptide wherein the HGPRBMY1  
polypeptide is HGPRBMY1 or a functionally equivalent derivative thereof, preferably  
wherein the subject is a human, wherein the HGPRBMY1 polypeptide is contained in  
30 a pharmaceutical composition.

The invention further relates to a method for the treatment of immune-related  
disorders, comprising modulating the activity of a HGPRBMY1 polypeptide.

The invention further relates to a method for the treatment of immune-related  
disorders, comprising modulating the activity of a HGPRBMY1 polypeptide, wherein  
35 the HGPRBMY1 polypeptide is HGPRBMY1 or a functionally equivalent derivative  
thereof.

The invention further relates to a method for the treatment of  
5 immune-related disorders, comprising modulating the activity of a HGPRBMY1  
polypeptide, wherein the HGPRBMY1 polypeptide is HGPRBMY1 or a functionally  
equivalent derivative thereof, wherein the method comprises administering an  
effective amount of a compound that agonizes or antagonizes the activity of the  
HGPRBMY1 polypeptide.

10 The invention further relates to a method for the treatment of immune-related  
disorders, comprising administering an effective amount of a compound that  
decreases expression of a HGPRBMY1 gene.

The invention further relates to a method for the treatment of  
immune-related disorders, comprising administering an effective amount of a  
15 compound that decreases expression of a HGPRBMY1 gene, wherein the compound  
is an oligonucleotide encoding an antisense or ribozyme molecule that targets  
HGPRBMY1 transcripts and inhibits translation.

The invention further relates to a method for the treatment of  
immune-related disorders, comprising administering an effective amount of a  
20 compound that decreases expression of a HGPRBMY1 gene, wherein the compound  
is an oligonucleotide that forms a triple helix with the promoter of the HGPRBMY1  
gene and inhibits transcription.

The invention further relates to a method for the treatment of  
immune-related disorders, comprising administering an effective amount of a  
25 compound that increases expression of a HGPRBMY1 gene.

The invention further relates to a pharmaceutical formulation for the  
treatment of immune-related disorders, comprising a compound that activates or  
inhibits HGPRBMY1 activity, mixed with a pharmaceutically acceptable carrier.

The invention further relates to a method for identifying compounds  
30 that modulate the activity of a G-protein coupled receptor comprising the step of  
(a) contacting a test compound to a cell that expresses a HGPRBMY1 gene and the  
G-protein coupled receptor, and measuring activity; (b) contacting a test  
compound to a cell that expresses a HGPRBMY1 gene but does not express the  
G-protein coupled receptor, and measuring activity; and (c) comparing activity  
35 obtained in (b) with the activity obtained in (a); such that if the level obtained in (b)  
differs from that obtained in (b), a compound that modulates G-protein coupled  
receptor activity is identified

The invention further relates to a method for identifying compounds  
5 that regulate heart-related disorders, comprising the step of contacting a test  
compound with a cell which expresses a nucleic acid of SEQ ID NO:13, and  
determining whether the test compound modulates HGPRBMY2 activity.

The invention further relates to a method for identifying compounds  
that regulate heart-related disorders comprising the step of contacting a test compound  
10 with a nucleic acid of SEQ ID NO:13; and determining whether the test compound  
interacts with the nucleic acid of SEQ ID NO:13.

The invention further relates to a method for identifying compounds  
that regulate heart-related disorders, comprising the step of contacting a test  
compound with a cell or cell lysate containing a reporter gene operatively associated  
15 with a HGPRBMY2 regulatory element; and detecting expression of the reporter gene  
product.

The invention further relates to a method for identifying compounds  
that regulate heart-related disorders comprising the step of contacting a test compound  
with a cell or cell lysate containing HGPRBMY2 transcripts; and detecting the  
20 translation of the HGPRBMY2 transcript.

The invention further relates to a method for modulating heart-related  
disorders in a subject, comprising administering to the subject a therapeutically  
effective amount of a HGPRBMY2 polypeptide.

The invention further relates to a method for modulating heart-related  
25 disorders in a subject, comprising administering to the subject a therapeutically  
effective amount of a HGPRBMY2 polypeptide wherein the HGPRBMY2  
polypeptide is HGPRBMY2 or a functionally equivalent derivative thereof, preferably  
wherein the subject is a human.

The invention further relates to a method for modulating heart-related  
30 disorders in a subject, comprising administering to the subject a therapeutically  
effective amount of a HGPRBMY2 polypeptide wherein the HGPRBMY2  
polypeptide is HGPRBMY2 or a functionally equivalent derivative thereof, preferably  
wherein the subject is a human, wherein the HGPRBMY2 polypeptide is contained in  
a pharmaceutical composition.

35 The invention further relates to a method for the treatment of heart-related  
disorders, comprising modulating the activity of a HGPRBMY2 polypeptide.

The invention further relates to a method for the treatment of heart-related disorders, comprising modulating the activity of a HGPRBMY2 polypeptide, wherein the HGPRBMY2 polypeptide is HGPRBMY2 or a functionally equivalent derivative thereof.

The invention further relates to a method for the treatment of heart-related disorders, comprising modulating the activity of a HGPRBMY2 polypeptide, wherein the HGPRBMY2 polypeptide is HGPRBMY2 or a functionally equivalent derivative thereof, wherein the method comprises administering an effective amount of a compound that agonizes or antagonizes the activity of the HGPRBMY2 polypeptide.

The invention further relates to a method for the treatment of heart-related disorders, comprising administering an effective amount of a compound that decreases expression of a HGPRBMY2 gene.

The invention further relates to a method for the treatment of heart-related disorders, comprising administering an effective amount of a compound that decreases expression of a HGPRBMY2 gene, wherein the compound is an oligonucleotide encoding an antisense or ribozyme molecule that targets HGPRBMY2 transcripts and inhibits translation.

The invention further relates to a method for the treatment of heart-related disorders, comprising administering an effective amount of a compound that decreases expression of a HGPRBMY2 gene, wherein the compound is an oligonucleotide that forms a triple helix with the promoter of the HGPRBMY2 gene and inhibits transcription.

The invention further relates to a method for the treatment of heart-related disorders, comprising administering an effective amount of a compound that increases expression of a HGPRBMY2 gene.

30 The invention further relates to a pharmaceutical formulation for the treatment of heart-related disorders, comprising a compound that activates or inhibits HGPRBMY2 activity, mixed with a pharmaceutically acceptable carrier.

The invention further relates to a method for identifying compounds that modulate the activity of a G-protein coupled receptor comprising the step of 35 (a) contacting a test compound to a cell that expresses a HGPRBMY2 gene and the G-protein coupled receptor, and measuring activity; (b) contacting a test compound to a cell that expresses a HGPRBMY2 gene but does not express the

G-protein coupled receptor, and measuring activity; and (c) comparing activity  
5 obtained in (b) with the activity obtained in (a); such that if the level obtained in (b)  
differs from that obtained in (b), a compound that modulates G-protein coupled  
receptor activity is identified

### 3.1 DEFINITIONS

10 The term "derivative" as used herein refers to a polypeptide that comprises an  
amino acid sequence of a GPCR polypeptide or peptide as described herein that has been  
altered by the introduction of amino acid residue substitutions, deletions or additions. The  
term "derivative" as used herein also refers to a GPCR polypeptide or peptide that has  
15 been modified, *i.e.*, by the covalent attachment of any type of molecule to the  
polypeptide. For example, but not by way of limitation, a GPCR polypeptide or peptide  
may be modified, *e.g.*, by glycosylation, acetylation, pegylation, phosphorylation,  
amidation, derivatization by known protecting/blocking groups, proteolytic cleavage,  
linkage to a cellular ligand or other polypeptide, etc. A derivative of a GPCR polypeptide  
20 or peptide may be modified by chemical modifications using techniques known to those  
of skill in the art, including, but not limited to specific chemical cleavage, acetylation,  
formylation, metabolic synthesis of tunicamycin, etc. Further, a derivative of a GPCR  
polypeptide or peptide may contain one or more non-classical amino acids. A polypeptide  
derivative possesses a similar or identical function as a GPCR polypeptide or peptide  
25 described herein.

An "isolated" or "purified" polypeptide or polypeptide complex of the invention  
is substantially free of cellular material or other contaminating polypeptides from the cell  
or tissue source from which the polypeptide is derived, or substantially free of chemical  
30 precursors or other chemicals when chemically synthesized. The language "substantially  
free of cellular material" includes preparations of a polypeptide or polypeptide complex  
in which the polypeptide or polypeptide complex is separated from cellular components  
of the cells from which it is isolated or recombinantly produced. Thus, a polypeptide or  
polypeptide complex that is substantially free of cellular material includes preparations  
35 of polypeptide or polypeptide complex having less than about 30%, 20%, 10%, or 5% (by  
dry weight) of a heterologous polypeptide (also referred to herein as a "contaminating  
polypeptide"). When the polypeptide or polypeptide complex is recombinantly produced,

it is also preferably substantially free of culture medium, *i.e.*, culture medium represents  
5 less than about 20%, 10%, or 5% of the volume of the polypeptide preparation. When the  
polypeptide or polypeptide complex is produced by chemical synthesis, it is preferably  
substantially free of chemical precursors or other chemicals, *i.e.*, it is separated from  
chemical precursors or other chemicals which are involved in the synthesis of the  
10 polypeptide. Accordingly such preparations of the polypeptide or polypeptide complex  
have less than about 30%, 20%, 10%, 5% (by dry weight) of chemical precursors or  
compounds other than the polypeptide or polypeptide complex of interest. In a preferred  
embodiment, polypeptides or polypeptide complexes or peptides of the invention are  
isolated or purified.

15 An "isolated" nucleic acid molecule is one which is separated from other nucleic  
acid molecules which are present in the natural source of the nucleic acid molecule.  
Moreover, an "isolated" nucleic acid molecule, such as a cDNA molecule, can be  
substantially free of other cellular material, or culture medium when produced by  
recombinant techniques, or substantially free of chemical precursors or other chemicals  
20 when chemically synthesized.

25 "Plasmids" are designated by a lower case p preceded and/or followed by capital  
letters and/or numbers. The starting plasmids herein are either commercially available,  
publicly available on an unrestricted basis, or can be constructed from available plasmids  
in accord with published procedures. In addition, equivalent plasmids to those described  
are known in the art and will be apparent to the ordinarily skilled artisan.

30 The term "fusion polypeptide" as used herein refers to a polypeptide that  
comprises an amino acid sequence of a polypeptide or peptide and an amino acid  
sequence of another polypeptide or peptide (*e.g.*, GPCR fused to an epitope tag such as  
a hexa-histidine motif, or a GPCR domain fused to another GPCR domain, such as two  
35 or more extracellular domains in tandem).

35 The term "GPCR antigen" refers to a GPCR polypeptide or peptide to which an  
antibody or antibody fragment immunospecifically binds. A GPCR antigen also refers to  
an analog or derivative of a GPCR polypeptide or peptide to which an antibody or  
antibody fragment immunospecifically binds.

The term "antibodies or antibody fragments that immunospecifically bind to a  
GPCR antigen" as used herein refers to antibodies, Fab's of antibodies, or other binding

portions of antibodies, that specifically bind to either a native and/or denatured GPCR  
5 polypeptide or a GPCR peptide and do not non-specifically bind to other polypeptides. Antibodies, or Fab portions thereof, that immunospecifically bind to a GPCR polypeptide or peptide may have cross-reactivity with other antigens. Preferably, antibodies or fragments that immunospecifically bind to a GPCR polypeptide or peptide do not cross-react with other antigens. Antibodies or fragments that immunospecifically bind to a  
10 GPCR polypeptide can be identified, for example, by immunoassays or other techniques known to those of skill in the art.

The term "patient in need thereof" refers to a human with, or at risk of, a disease or disorder associated with the gene or gene product of the invention. Further this term  
15 includes in certain embodiments immunocompromised patients. For research purposes, an animal model, for example a mouse model or monkey model, can be utilized to simulate such a patient in some circumstances.

To determine the percent identity of two amino acid sequences or of two nucleic acid sequences, the sequences are aligned for optimal comparison purposes (e.g., gaps  
20 can be introduced in the sequence of a first amino acid or nucleic acid sequence for optimal alignment with a second amino acid or nucleic acid sequence). The amino acid residues or nucleic acids at corresponding amino acid positions or nucleic acid positions are then compared. When a position in the first sequence is occupied by the same amino acid residue or nucleic acid as the corresponding position in the second sequence, then  
25 the molecules are identical at that position. The percent identity between the two sequences is a function of the number of identical positions shared by the sequences (i.e., % identity = number of identical overlapping positions/total number of positions x 100%). In one embodiment, the two sequences are the same length.

30 The determination of percent identity between two sequences can also be accomplished using a mathematical algorithm. A preferred, non-limiting example of a mathematical algorithm utilized for the comparison of two sequences is the algorithm of Karlin and Altschul, 1990, Proc. Natl. Acad. Sci. U.S.A. 87:2264-2268, modified as in Karlin and Altschul, 1993, Proc. Natl. Acad. Sci. U.S.A. 90:5873-5877. Such an  
35 algorithm is incorporated into the NBLAST and XBLAST programs of Altschul et al., 1990, J. Mol. Biol. 215:403. BLAST nucleic acid searches can be performed with the NBLAST nucleic acid program parameters set, e.g., for score=100, wordlength=12 to

obtain nucleic acid sequences homologous to a nucleic acid molecules of the present invention. BLAST polypeptide searches can be performed with the XBLAST program parameters set, *e.g.*, to score-50, wordlength=3 to obtain amino acid sequences homologous to a polypeptide molecule of the present invention. To obtain gapped alignments for comparison purposes, Gapped BLAST can be utilized as described in 5 Altschul et al., 1997, Nucleic Acids Res. 25:3389-3402. Alternatively, PSI-BLAST can be used to perform an iterated search which detects distant relationships between 10 molecules (*Id.*). When utilizing BLAST, Gapped BLAST, and PSI-Blast programs, the default parameters of the respective programs (*e.g.*, of XBLAST and NBLAST) can be used (*e.g.*, <http://www.ncbi.nlm.nih.gov>). Another preferred, non-limiting example of a 15 mathematical algorithm utilized for the comparison of sequences is the algorithm of Myers and Miller, 1988, CABIOS 4:11-17. Such an algorithm is incorporated in the ALIGN program (version 2.0) which is part of the GCG sequence alignment software package. When utilizing the ALIGN program for comparing amino acid sequences, a 20 PAM120 weight residue table, a gap length penalty of 12, and a gap penalty of 4 can be used.

The percent identity between two sequences can be determined using techniques similar to those described above, with or without allowing gaps. In calculating percent identity, typically only exact matches are counted.

25

#### 4. DESCRIPTION OF THE FIGURES

The file of this patent contains at least one Figure executed in color. Copies of this patent with color Figure(s) will be provided by the Patent and Trademark Office upon 30 request and payment of the necessary fee.

Figure 1: Nucleic acid sequence of the coding region of HGPRBMY1. The 5' untranslated region is the first group of sequences, the second group of sequences is the open reading frame of HGPRBMY1 and the third set is the 3' untranslated region.

Figure 2: Theoretical translation of the open reading frame of the cDNA of Figure 35 1, resulting in the polypeptide sequence of HGPRBMY1.

Figure 3: The shaded sequences in the polypeptide sequence in the upper half of 5 the figure reflect the transmembrane regions. The bottom of the figure depicts a hydrophathy plot of the polypeptide sequence of Figure 2.

Figure 4: Sequence alignment of HGPRBMY1 and related G-protein coupled receptors. The GCG pileup program was used to generate the alignment. The blackened 10 areas represent identical amino acids in more than half of the listed sequences and the grey highlighted areas represent similar amino acids.

Figure 5: Expression profile of HGPRBMY1 in various tissues as measured by PCR. The PCR data was converted into a relative assessment of the difference in transcript abundance amongst the tissues tested. Transcripts corresponding to the orphan 15 GPCR, HGPRBMY1, are expressed most highly in bone marrow, spleen and thymus.

Figure 6: Nucleic acid sequence of the coding region of HGPRBMY2. The 5' untranslated region is the first group of sequences, the second group of sequences is the open reading frame of HGPRBMY2 and the third set is the 3' untranslated region.

Figure 7: Theoretical translation of the open reading frame of the cDNA of Figure 20 Figure 6, resulting in the polypeptide sequence of HGPRBMY2.

Figure 8: The shaded sequences in the polypeptide sequence in the upper half of the figure reflect the transmembrane regions. The bottom of the figure depicts a hydrophathy plot of the polypeptide sequence of Figure 7.

Figure 9: Sequence alignment of HGPRBMY2 and related G-protein coupled 25 receptors. The GCG pileup program was used to generate the alignment. The blackened areas represent identical amino acids in more than half of the listed sequences and the grey highlighted areas represent similar amino acids.

Figure 10: Expression profile of HGPRBMY2 in various tissues as measured by 30 PCR. The PCR data was converted into a relative assessment of the difference in transcript abundance amongst the tissues tested. Transcripts corresponding to the orphan GPCR, HGPRBMY2, are expressed most highly in testis, heart, and thymus.

Figure 11: Untransfected Cho NFAT-CRE cell line FACS profile. Control Cho- 35 NFAT/CRE (Nuclear Factor Activator of Transcription (NFAT) / cAMP response element (CRE)) cell lines were incubated with 10 nM PMA and 1 uM Thapsigargin / 10 uM Forskolin, respectively, in the absence of the pcDNA3.1 Hygro<sup>TM</sup> / HGPRBMY2 mammalian expression vector transfection, as described herein. The stimulated cells were

sorted via FACS (Fluorescent Assisted Cell Sorter) according to their wavelength 5 emission at 518 nM (Channel R3 - Green Cells), and 447 nM (Channel R2 – Blue Cells). As shown, the vast majority of cells emit at 518 nM, with minimal emission observed at 10 447 nM. The latter is expected since the NFAT/CRE response elements remain dormant in the absence of an activated G-protein dependent signal transduction pathway (e.g., pathways mediated by Gq/11 or Gs coupled receptors). As a result, the cell permeant, CCF2/AM<sup>TM</sup> (Aurora Biosciences; Zlokarnik, et al., 1998) substrate remains intact and emits light at 518 nM.

Figure 12: Overexpression Of BMY2 Constitutively Couples Through The NFAT/CRE Response Element. Cho-NFAT/CRE cell lines transfected with the 15 pcDNA3.1 Hygro<sup>TM</sup> / HGPRBMY2 mammalian expression vector were incubated with 10 nM PMA and 1 uM Thapsigargin / 10 uM Forskolin, respectively, as described herein. The stimulated cells were sorted via FACS according to their wavelength emission at 518 20 nM (Channel R3 - Green Cells), and 447 nM (Channel R2 – Blue Cells). As shown, overexpression of HGPRBMY2 results in functional coupling and subsequent activation of beta lactamase gene expression, as evidenced by the significant number of cells with 25 fluorescent emission at 447 nM relative to the non-transfected control Cho-NFAT/CRE cells (shown in Figure 11).

Figure 13: HGPRBMY2 Does Not Couple Through The cAMP Response 25 Element. HEK-CRE cell lines transfected with the pcDNA3.1 Hygro<sup>TM</sup> / HGPRBMY2 mammalian expression vector were incubated with 10 nM PMA and 10 uM Forskolin, as described herein. The stimulated cells were sorted via FACS according to their wavelength emission at 518 nM (Channel R3 - Green Cells), and 447 nM (Channel R2 – Blue Cells). As shown, overexpression of HGPRBMY2 in the HEK-CRE cells did not 30 result in functional coupling, as evidenced by the insignificant background level of cells with fluorescent emission at 447 nM.

Figure 14: Expressed HGPRBMY2 Localizes To The Plasma Membrane. Cho- 35 NFAT/CRE cell lines transfected with the pcDNA3.1 Hygro<sup>TM</sup> / HGPRBMY2-FLAG mammalian expression vector were subjected to immunocytochemistry using an FITC conjugated Anti Flag monoclonal antibody, as described herein. Panel A shows the transfected Cho-NFAT/CRE cells under visual wavelengths, and panel B shows the fluorescent emission of the same cells at 530 nm after illumination with a laser at 447

nm. The plasma membrane localization is clearly evident in panel B, and is consistent  
5 with the HGPRBMY2 polypeptide representing a member of the GPCR family.

Figure 15: Transfected Cho-NFAT/CRE cell lines With Intermediate and High  
Beta Lactamase Expression Levels Useful In Screens to Identify HGPRBMY2 Agonists  
and/or Antagonists. Several Cho-NFAT/CRE cell lines transfected with the pcDNA3.1  
10 Hygro <sup>TM</sup> / HGPRBMY2 mammalian expression vector were isolated via FACS that had  
either intermediate or high beta lactamase expression levels post stimulation with 10 nM  
PMA and 1 uM Thapsigargin / 10 uM Forskolin, as described herein. Panel A shows  
HGPRBMY2 transfected Cho-NFAT/CRE cells prior to stimulation with 10 nM PMA  
15 and 1 uM Thapsigargin / 10 uM Forskolin ( - P/T/F). Panel B shows HGPRBMY2  
transfected Cho-NFAT/CRE cells after stimulation with 10 nM PMA and 1 uM  
Thapsigargin / 10 uM Forskolin ( + P/T/F). Panel C shows HGPRBMY2 transfected Cho-  
20 NFAT/CRE cells after stimulation with 10 nM PMA and 1 uM Thapsigargin / 10 uM  
Forskolin ( + P/T/F) that have an intermediate level of beta lactamase expression. Panel  
D shows HGPRBMY2 transfected Cho-NFAT/CRE cells after stimulation with 10 nM  
PMA and 1 uM Thapsigargin / 10 uM Forskolin ( + P/T/F) that have a high level of beta  
lactamase expression.

Figure 16: Expanded Expression Profile Of The Novel Human G-Protein  
Coupled Receptor, HGPRBMY2. The figure illustrates the relative expression level of  
HGPRBMY2 amongst various mRNA tissue sources. As shown, the HGPRBMY2  
25 polypeptide was predominately expressed in the heart, with highest expression in the left  
ventricle, significantly in tissues of the posterior hypothalamus (1000-fold greater than  
most other tissues), the DRG, and to a lesser extent in tissues throughout the brain in  
addition to other tissues as shown. Expression data was obtained by measuring the steady  
30 state HGPRBMY2 mRNA levels by quantitative PCR using the PCR primer pair  
provided as SEQ ID NO:25 and 26, and Taqman probe (SEQ ID NO:27) as described  
herein.

## 35 5. DETAILED DESCRIPTION OF THE INVENTION

HGPRBMY1 is a novel receptor expressed in bone marrow, spleen and thymus.  
The present invention use of HGPRBMY1 nucleic acids, HGPRBMY1 polypeptides and  
peptides, as well as antibodies to the HGPRBMY1 (which can, for example, act as

5 detection of mutant HGPRBMY1 or inappropriately expressed HGPRBMY1, particularly  
for the diagnosis of immune disorders either related to HGPRBMY1 expression,  
activation or down regulation, or wherein HGPRBMY1 serves as an indicator of an  
immune disorder. The HGPRBMY1 polypeptides, HGPRBMY1 fusion polypeptides,  
10 HGPRBMY1 nucleic acid sequences, host cell expression systems, antibodies,  
antagonists, agonists and genetically engineered cells and animals can be used for  
screening for drugs effective in the treatment of such immune disorders. The use of  
engineered host cells and/or animals may offer an advantage in that such systems allow  
not only for the identification of compounds that bind to the ECD or to the CD of the  
15 HGPRBMY1, and/or can be used to identify compounds that modulate the signal  
transduced by the activated HGPRBMY1.

Finally, the HGPRBMY1 polypeptide products (especially derivatives such as  
peptides corresponding to a HGPRBMY1 ECD, or truncated polypeptides lacking a  
hydrophobic TM domain, which are soluble under normal physiological conditions) and  
fusion polypeptide products (especially HGPRBMY1-Ig fusion polypeptides, *i.e.*, fusions  
20 of a domain of HGPRBMY1, *e.g.*, ECD,  $\Delta$ TM or CD to a heterologous sequence such  
as IgFc), antibodies (including fragments thereof), antagonists or agonists (including  
compounds that modulate signal transduction which may act on downstream targets in  
the HGPRBMY1 signal transduction pathway) can be used for therapy of such diseases.  
25 For example, the administration of an effective amount of a pharmaceutical composition  
comprising a soluble ECD, CD,  $\Delta$ TM, CD-IgFc fusion, ECD-IgFc fusion polypeptide or  
an antibody (or fragment thereof) that mimics the HGPRBMY1 ECD would modulate  
HGPRBMY1 activity, leading to prevention or treatment of an immune disorder.

Nucleic acid constructs encoding the HGPRBMY1 products above can be used  
30 to engineer host cells to express such HGPRBMY1 products *in vivo*. These implanted  
cells, when implanted into a host, deliver a continuous supply of a soluble ECD or a  
fusion polypeptide that modulates HGPRBMY1 activity. Nucleic acid constructs  
encoding functional HGPRBMY1, mutant HGPRBMY1, as well as antisense and  
35 ribozyme molecules can be used in gene therapy for the modulation of HGPRBMY1  
expression and/or activity in the treatment of immune disorders. Thus, the invention  
features pharmaceutical formulations and methods for treating immune disorders.

The strong homology to human G-protein coupled receptors, combined with the

5 HGPRBMY1 agonists or antagonists), antagonists that inhibit receptor activity or expression, or agonists that activate receptor activity or increase its expression in the diagnosis and treatment of immune disorders, including, but not limited to immune disorders in animals, including humans. The diagnosis of abnormality associated with HGPRBMY1 in a patient, or an abnormality in the HGPRBMY1 signal transduction pathway, will assist in devising a proper treatment or therapeutic regimen. In addition, 10 HGPRBMY1 nucleic acids and HGPRBMY1 polypeptides are useful for the identification of compounds effective in the treatment of immune disorders regulated by HGPRBMY1.

15 The invention features HGPRBMY1 polypeptides or portions of the full length polypeptide, *i.e.*, peptides, which can be designed to correspond to functional domains of the HGPRBMY1 (*e.g.*, full length polypeptide, ECD, TM or CD), or mutated, truncated or deleted HGPRBMY1 (*e.g.* an HGPRBMY1 with one or more functional domains or portions thereof deleted, such as  $\Delta$ TM and/or  $\Delta$ CD), or HGPRBMY1 fusion polypeptides (*e.g.* an HGPRBMY1 or a functional domain of HGPRBMY1, such as an 20 ECD fused to an unrelated polypeptide or peptide such as an immunoglobulin constant region, *i.e.*, IgFc), nucleic acid sequences encoding such products, and host cell expression systems that can produce such HGPRBMY1 products.

25 The invention also features antibodies and anti-idiotypic antibodies (including antibody fragments), antagonists and agonists of the HGPRBMY1, as well as compounds or nucleic acid constructs that inhibit expression of the HGPRBMY1 gene (transcription factor inhibitors, antisense and ribozyme molecules, or gene or regulatory sequence replacement constructs), or promote expression of HGPRBMY1 (*e.g.*, expression constructs in which HGPRBMY1 coding sequences are operatively associated with 30 expression control elements such as promoters, promoter/enhancers, etc.).

35 The invention also features host cells or animals genetically engineered to express exogenous HGPRBMY1 (or mutants thereof), cells or animals engineered to increase expression of the endogenous HGPRBMY1, cells or animals engineered to express a mutated HGPRBMY1, or cells or animals engineered to inhibit expression of either an animal's endogenous HGPRBMY1.

The HGPRBMY1 polypeptides, HGPRBMY1 fusion polypeptides, HGPRBMY1 nucleic acid sequences, antibodies, antagonists and agonists can be useful for the

predominate localized expression in bone marrow and spleen, in conjunction with the p27  
5 and IkB association, suggests the HGPRBMY1 polynucleotides and polypeptides may  
be useful in treating, diagnosing, prognosing, and/or preventing immune diseases and/or  
disorders. Representative uses are described elsewhere herein. Briefly, the strong  
expression in immune tissue indicates a role in regulating the proliferation; survival;  
10 differentiation; and/or activation of hematopoietic cell lineages, including blood stem  
cells.

The HGPRBMY1 polypeptide may also be useful as a preventative agent for  
immunological disorders including arthritis, asthma, immunodeficiency diseases such as  
AIDS, leukemia, rheumatoid arthritis, granulomatous disease, inflammatory bowel  
15 disease, sepsis, acne, neutropenia, neutrophilia, psoriasis, hypersensitivities, such as T-  
cell mediated cytotoxicity; immune reactions to transplanted organs and tissues, such as  
host-versus-graft and graft-versus-host diseases, or autoimmunity disorders, such as  
autoimmune infertility, lense tissue injury, demyelination, systemic lupus erythematosus,  
drug induced hemolytic anemia, rheumatoid arthritis, Sjogren's disease, and scleroderma.  
20 The HGPRBMY1 polypeptide may be useful for modulating cytokine production, antigen  
presentation, or other processes, such as for boosting immune responses, etc.

Moreover, the protein may represent a factor that influences the differentiation  
or behavior of other blood cells, or that recruits hematopoietic cells to sites of injury.  
25 Thus, this gene product is thought to be useful in the expansion of stem cells and  
committed progenitors of various blood lineages, and in the differentiation and/or  
proliferation of various cell types. Furthermore, the protein may also be used to determine  
biological activity, raise antibodies, as tissue markers, to isolate cognate ligands or  
receptors, to identify agents that modulate their interactions, in addition to its use as a  
30 nutritional supplement. Protein, as well as, antibodies directed against the protein may  
show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Various aspects of the invention are described in greater detail in the subsections  
below.

35 HGPRBMY2, described for the first time herein, is a novel receptor protein  
expressed in heart, brain tissues, testis, and thymus tissues. The invention encompasses  
the use of HGPRBMY2 nucleic acids, HGPRBMY2 proteins and peptides, as well as  
antibodies to the HGPRBMY2 (which can, for example, act as HGPRBMY2 agonists or

antagonists), antagonists that inhibit receptor activity or expression, or agonists that 5 activate receptor activity or increase its expression in the diagnosis and treatment of cardiovascular disorders, including, but not limited to heart disease in animals, including humans. The diagnosis of an HGPRBMY2 abnormality in a patient, or an abnormality 10 in the HGPRBMY2 signal transduction pathway, will assist in devising a proper treatment or therapeutic regimen. In addition, HGPRBMY2 nucleic acids and HGPRBMY2 proteins are useful for the identification of compounds effective in the treatment of cardiovascular disorders regulated by the HGPRBMY2.

Expanded analysis of HGPRBMY2 expression levels by TaqMan™ quantitative PCR (see Figure 16) confirmed that the HGPRBMY2 polypeptide is expressed at very 15 low levels in heart and testis, with relatively low-level expression in the brain sub regions tested as shown using the SYBR green experiments (see Figure 10). HGPRBMY2 mRNA was expression predominately in heart, with the highest concentration in the left ventricle, and the posterior hypothalamus; significantly in the DRG and other tissues 20 throughout the brain, and to a lesser extent in the spinal cord in adition to other tissues as shown. These data suggest that HGPRBMY2 may be useful for the treatment and/or amelioration of metabolic disorders, mainly obesity, and for the treatment of pain disorders.

The strong homology to human G-protein coupled receptors, combined with the 25 predominate localized expression in heart tissue suggests the HGPRBMY2 polynucleotides and polypeptides may be useful in treating, diagnosing, prognosing, and/or preventing cardiovascular diseases and/or disorders, which include, but are not limited to: myocardio infarction, congestive heart failure, arrhythmias, cardiomyopathy, atherosclerosis, arterialsclerosis, microvascular disease, embolism, thromobosis, 30 pulmonary edema, palpitation, dyspnea, angina, hypotension, syncope, heart murmer, aberrant ECG, hypertrophic cardiomyopathy, the Marfan syndrome, sudden death, prolonged QT syndrome, congenital defects, cardiac viral infections, valvular heart disease, and hypertension.

35 Similarly, HGPRBMY2 polynucleotides and polypeptides may be useful for ameliorating cardiovascular diseases and symptoms which result indirectly from various non-cardiavascular effects, which include, but are not limited to, the following, obesity, smoking, Down syndrome (associated with endocardial cushion defect); bony

abnormalities of the upper extremities (associated with atrial septal defect in the Holt-  
5 Oram syndrome); muscular dystrophies (associated with cardiomyopathy);  
hemochromatosis and glycogen storage disease (associated with myocardial infiltration  
and restrictive cardiomyopathy); congenital deafness (associated with prolonged QT  
interval and serious cardiac arrhythmias); Raynaud's disease (associated with primary  
10 pulmonary hypertension and coronary vasospasm); connective tissue disorders, i.e., the  
Marfan syndrome, Ehlers-Danlos and Hurler syndromes, and related disorders of  
mucopolysaccharide metabolism (aortic dilatation, prolapsed mitral valve, a variety of  
arterial abnormalities); acromegaly (hypertension, accelerated coronary atherosclerosis,  
conduction defects, cardiomyopathy); hyperthyroidism (heart failure, atrial fibrillation);  
15 hypothyroidism (pericardial effusion, coronary artery disease); rheumatoid arthritis  
(pericarditis, aortic valve disease); scleroderma (cor pulmonale, myocardial fibrosis,  
pericarditis); systemic lupus erythematosus (valvulitis, myocarditis, pericarditis);  
sarcoidosis (arrhythmias, cardiomyopathy); postmenopausal effects, Chlamydial  
infections, polycystic ovary disease, thyroid disease, alcoholism, diet, and exfoliative  
20 dermatitis (high-output heart failure), for example.

Moreover, polynucleotides and polypeptides, including fragments and/or  
antagonists thereof, have uses which include, directly or indirectly, treating, preventing,  
diagnosing, and/or prognosing the following, non-limiting, cardiovascular infections:  
25 blood stream invasion, bacteremia, sepsis, *Streptococcus pneumoniae* infection, group  
a streptococci infection, group b streptococci infection, *Enterococcus* infection,  
nonenterococcal group D streptococci infection, nonenterococcal group C streptococci  
infection, nonenterococcal group G streptococci infection, *Streptoccus viridans* infection,  
30 *Staphylococcus aureus* infection, coagulase-negative staphylococci infection, gram-  
negative *Bacilli* infection, *Enterobacteriaceae* infection, *Pseudomonas* spp. Infection,  
*Acinobacter* spp. Infection, *Flavobacterium meningosepticum* infection, *Aeromonas* spp.  
Infection, *Stenotrophomonas maltophilia* infection, gram-negative coccobacilli infection,  
*Haemophilus influenza* infection, *Branhamella catarrhalis* infection, anaerobe infection,  
35 *Bacteroides fragilis* infection, *Clostridium* infection, fungal infection, *Candida* spp.  
Infection, non-albicans *Candida* spp. Infection, *Hansenula anomala* infection, *Malassezia*  
*furfur* infection, nontuberculous *Mycobacteria* infection, *Mycobacterium avium* infection,  
*Mycobacterium chelonae* infection, *Mycobacterium fortuitum* infection, spirochetal

infection, *Borrelia burgdorferi* infection, in addition to any other cardiovascular disease 5 and/or disorder (e.g., non-sepsis) implicated by the causative agents listed above or elsewhere herein.

The strong homology to human G-protein coupled receptor proteins, combined with the localized expression in various brain tissues suggests HGPRBMY2 10 polynucleotides and polypeptides may be useful in treating, diagnosing, prognosing, and/or preventing neurodegenerative disease states, behavioral disorders, or inflammatory 15 conditions. Representative uses are described in the section 5.6c below, in the Examples, and elsewhere herein. Briefly, the uses include, but are not limited to the detection, treatment, and/or prevention of Alzheimer's Disease, Parkinson's Disease, Huntington's 20 Disease, Tourette Syndrome, meningitis, encephalitis, demyelinating diseases, peripheral neuropathies, neoplasia, trauma, congenital malformations, spinal cord injuries, ischemia and infarction, aneurysms, hemorrhages, schizophrenia, mania, dementia, paranoia, obsessive compulsive disorder, depression, panic disorder, learning disabilities, ALS, 25 psychoses, autism, and altered behaviors, including disorders in feeding, sleep patterns, balance, and perception. In addition, elevated expression of this gene product in regions of the brain indicates it plays a role in normal neural function. Potentially, this gene product is involved in synapse formation, neurotransmission, learning, cognition, homeostasis, or neuronal differentiation or survival. Furthermore, the protein may also 25 be used to determine biological activity, to raise antibodies, as tissue markers, to isolate cognate ligands or receptors, to identify agents that modulate their interactions, in addition to its use as a nutritional supplement. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

30 Alternatively, the strong homology to G-protein coupled receptors, combined with the predominate localized expression in testis tissue suggests the potential utility for HGPRBMY2 polynucleotides and polypeptides in treating, diagnosing, prognosing, and/or preventing testicular, in addition to reproductive disorders.

35 In preferred embodiments, HGPRBMY2 polynucleotides and polypeptides including agonists and fragments thereof, have uses which include treating, diagnosing, prognosing, and/or preventing the following, non-limiting, diseases or disorders of the

5 testis: spermatogenesis, infertility, Klinefelter's syndrome, XX male, epididymitis, genital  
warts, germinal cell aplasia, cryptorchidism, varicocele, immotile cilia syndrome, and  
viral orchitis. The HGPRBMY2 polynucleotides and polypeptides including agonists and  
fragments thereof, may also have uses related to modulating testicular development,  
embryogenesis, reproduction, and in ameliorating, treating, and/or preventing testicular  
10 proliferative disorders (e.g., cancers, which include, for example, choriocarcinoma,  
Nonseminoma, seminoma, and testicular germ cell tumors).

15 Likewise, the predominate localized expression in testis tissue also emphasizes  
the potential utility for HGPRBMY2 polynucleotides and polypeptides in treating,  
diagnosing, prognosing, and/or preventing metabolic diseases and disorders which  
15 include the following, not limiting examples: premature puberty, incomplete puberty,  
Kallman syndrome, Cushing's syndrome, hyperprolactinemia, hemochromatosis,  
congenital adrenal hyperplasia, FSH deficiency, and granulomatous disease, for example.

20 This gene product may also be useful in assays designed to identify binding  
agents, as such agents (antagonists) are useful as male contraceptive agents. The testes  
are also a site of active gene expression of transcripts that is expressed, particularly at low  
levels, in other tissues of the body. Therefore, this gene product may be expressed in  
other specific tissues or organs where it may play related functional roles in other  
processes, such as hematopoiesis, inflammation, bone formation, and kidney function,  
25 to name a few possible target indications.

30 The strong homology to G-protein coupled receptors, combined with the localized  
expression in thymus tissue suggests the HGPRBMY2 polynucleotides and polypeptides  
may be useful in treating, diagnosing, prognosing, and/or preventing immune diseases  
and/or disorders. The strong expression in immune tissue indicates a role in regulating  
the proliferation; survival; differentiation; and/or activation of hematopoietic cell  
lineages, including blood stem cells.

35 The HGPRBMY2 polypeptide may also be useful as a preventative agent for  
immunological disorders including arthritis, asthma, immunodeficiency diseases such as  
AIDS, leukemia, rheumatoid arthritis, granulomatous disease, inflammatory bowel  
disease, sepsis, acne, neutropenia, neutrophilia, psoriasis, hypersensitivities, such as T-  
cell mediated cytotoxicity; immune reactions to transplanted organs and tissues, such as

host-versus-graft and graft-versus-host diseases, or autoimmunity disorders, such as  
5 autoimmune infertility, lense tissue injury, demyelination, systemic lupus erythematosis, drug induced hemolytic anemia, rheumatoid arthritis, Sjogren's disease, and scleroderma. The HGPRBMY2 polypeptide may be useful for modulating cytokine production, antigen presentation, or other processes, such as for boosting immune responses, etc.

10 Moreover, the protein may represent a factor that influences the differentiation or behavior of other blood cells, or that recruits hematopoietic cells to sites of injury. Thus, this gene product is thought to be useful in the expansion of stem cells and committed progenitors of various blood lineages, and in the differentiation and/or proliferation of various cell types. Furthermore, the protein may also be used to determine 15 biological activity, raise antibodies, as tissue markers, to isolate cognate ligands or receptors, to identify agents that modulate their interactions, in addition to its use as a nutritional supplement. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

20 The invention features HGPRBMY2 polypeptides or portions of the full length polypeptide, *i.e.*, peptides, which can be designed to correspond to functional domains of the HGPRBMY2 (*e.g.*, full length protein, ECD, TM or CD), or mutated, truncated or deleted HGPRBMY2 (*e.g.* an HGPRBMY2 with one or more functional domains or portions thereof deleted, such as  $\Delta$ TM and/or  $\Delta$ CD), or HGPRBMY2 fusion polypeptides 25 (*e.g.* an HGPRBMY2 or a functional domain of HGPRBMY2, such as an ECD fused to an unrelated polypeptide or peptide such as an immunoglobulin constant region, *i.e.*, IgFc), nucleic acid sequences encoding such products, and host cell expression systems that can produce such HGPRBMY2 products.

30 The invention also features antibodies and anti-idiotypic antibodies (including Fab fragments), antagonists and agonists of the HGPRBMY2, as well as compounds or nucleic acid constructs that inhibit expression of the HGPRBMY2 gene (transcription factor inhibitors, antisense and ribozyme molecules, or gene or regulatory sequence replacement constructs), or promote expression of HGPRBMY2 (*e.g.*, expression constructs in which HGPRBMY2 coding sequences are operatively associated with 35 expression control elements such as promoters, promoter/enhancers, etc.). The invention also relates to host cells and animals genetically engineered to express the human

5 HGPRBMY2 (or mutants thereof) or to inhibit or "knock-out" expression of the animal's  
5 endogenous HGPRBMY2.

10 The HGPRBMY2 polypeptides or peptides, HGPRBMY2 fusion polypeptides, HGPRBMY2 nucleic acid sequences, antibodies, antagonists and agonists can be useful for the detection of mutant HGPRBMY2 or inappropriately expressed HGPRBMY2 for the diagnosis of cardiovascular disorders. The HGPRBMY2 polypeptides or peptides, HGPRBMY2 fusion polypeptides, HGPRBMY2 nucleic acid sequences, host cell expression systems, antibodies, antagonists, agonists and genetically engineered cells and animals can be used for screening for drugs effective in the treatment of such cardiovascular disorders. The use of engineered host cells and/or animals may offer an 15 advantage in that such systems allow not only for the identification of compounds that bind to the ECD of the HGPRBMY2, but can also identify compounds that affect the signal transduced by the activated HGPRBMY2.

20 Finally, the HGPRBMY2 protein products (especially soluble derivatives such as peptides corresponding to a HGPRBMY2 ECD, or truncated polypeptides lacking a hydrophobic TM domain) and fusion polypeptide products (especially HGPRBMY2-Ig fusion polypeptides, *i.e.*, fusions of the HGPRBMY2 or a domain of the HGPRBMY2, e.g., ECD,  $\Delta$ TM, or CD to a heterologous sequence such as IgFc), antibodies and anti-idiotypic antibodies (including Fab fragments), antagonists or agonists (including 25 compounds that modulate signal transduction which may act on downstream targets in the HGPRBMY2 signal transduction pathway) can be used for therapy of such diseases. For example, the administration of an effective amount of a pharmaceutical composition comprising a soluble HGPRBMY2 ECD,  $\Delta$ TM HGPRBMY2 or an ECD-IgFc fusion polypeptide or an anti-idiotypic antibody (or its Fab) that mimics the HGPRBMY2 ECD 30 would modulate activation of the GPCR by endogenous agonist or antagonist, and prevent or reduce binding and receptor activation, leading to heart failure.

35 Nucleic acid constructs encoding such HGPRBMY2 products can be used to genetically engineer host cells to express such HGPRBMY2 products *in vivo*; these genetically engineered cells function in the body delivering a continuous supply of the HGPRBMY2, HGPRBMY2 peptide, soluble ECD or  $\Delta$ TM or HGPRBMY2 fusion polypeptide that will modulate agonist or antagonist. Nucleic acid constructs encoding functional HGPRBMY2, mutant HGPRBMY2, as well as antisense and ribozyme

5 molecules can be used in "gene therapy" approaches for the modulation of HGPRBMY2 expression and/or activity in the treatment of cardiovascular disorders. Thus, the invention also encompasses pharmaceutical formulations and methods for treating cardiovascular disorders.

10 The invention is based, in part, on the surprising discovery of a receptor for agonist or antagonist expressed at significant concentration in heart and thymus. Various aspects of the invention are described in greater detail in the subsections below.

### 5.1. HGPRBMY1 Nucleic Acids

15 The cDNA sequence of HGPRBMY1 (SEQ ID NO:1) is 1554 base pairs long and is shown in Figure 1. The first set of sequence is the 5' untranslated, the second set is the open reading frame and the third set is the 5' untranslated. The open reading frame extends from nucleotides 247 to 1323 of SEQ ID NO:1. The deduced amino acid sequence encoded by the open reading frame of the cDNA of HGPRBMY1 is 359 amino acids (SEQ ID NO:2) and is shown in Figure 2.

20 The cDNA sequence of HGPRBMY2 (SEQ ID NO:13) is 2448 base pairs long and is shown in Figure 6. The first set of sequence is the 5' untranslated, the second set is the open reading frame and the third set is the 5' untranslated. The open reading frame extends from nucleotides 359 to 1651 of SEQ ID NO:13. The deduced amino acid sequence encoded by the open reading frame of the cDNA of HGPRBMY2 is 431 amino acids (SEQ ID NO:14) and is shown in Figure 7.

25 HGPRBMY1 nucleic acid sequences of the invention include: (a) the DNA sequence shown in SEQ ID NO:1; (b) nucleic acid sequence that encodes the polypeptide shown in SEQ ID NO:2; (c) any nucleic acid sequence that hybridizes to the complement 30 of the DNA sequence shown in SEQ ID NO:1 under highly stringent conditions, *e.g.*, hybridization to filter-bound DNA in 0.5 M NaHPO<sub>4</sub>, 7% sodium dodecyl sulfate (SDS), 1 mM EDTA at 65°C, and washing in 0.1x SSC/0.1% SDS at 68°C (Ausubel F. M. et al., eds., 1989, *Current Protocols in Molecular Biology*, Vol. I, Green Publishing Associates, Inc., and John Wiley & sons, Inc., New York, at p. 2.10.3) and encodes a functionally 35 equivalent gene product; and (d) any nucleic acid sequence that hybridizes to the complement of the DNA sequences that encode the amino acid sequence shown in SEQ ID NO:2 contained in cDNA clone as deposited with the ATCC® under less stringent

conditions, such as moderately stringent conditions, *e.g.*, washing in 0.2x SSC/0.1% SDS at 42°C (Ausubel et al., 1989, *supra*), yet which still encodes a functionally equivalent HGPRBMY1 gene product.

HGPRBMY2 nucleic acid sequences of the invention include: (a) the DNA sequence shown in SEQ ID NO:13; (b) nucleic acid sequence that encodes the polypeptide shown in SEQ ID NO:14; (c) any nucleic acid sequence that hybridizes to the complement of the DNA sequence shown in SEQ ID NO:13 under highly stringent conditions, *e.g.*, hybridization to filter-bound DNA in 0.5 M NaHPO<sub>4</sub>, 7% sodium dodecyl sulfate (SDS), 1 mM EDTA at 65°C, and washing in 0.1x SSC/0.1% SDS at 68°C (Ausubel F. M. et al., eds., 1989, *Current Protocols in Molecular Biology*, Vol. I, Green Publishing Associates, Inc., and John Wiley & sons, Inc., New York, at p. 2.10.3) and encodes a functionally equivalent gene product; and (d) any nucleic acid sequence that hybridizes to the complement of the DNA sequences that encode the amino acid sequence shown in SEQ ID NO:14 contained in cDNA clone as deposited with the ATCC® under less stringent conditions, such as moderately stringent conditions, *e.g.*, washing in 0.2x SSC/0.1% SDS at 42°C (Ausubel et al., 1989, *supra*), yet which still encodes a functionally equivalent HGPRBMY2 gene product.

Functional equivalents of the HGPRBMY1 include naturally occurring HGPRBMY1 present in other species, *i.e.*, orthologs, and mutant HGPRBMY1 whether naturally occurring or engineered. The invention also includes degenerate variants of sequences (a) through (d), *supra*. The invention also includes nucleic acid molecules, preferably DNA molecules, that hybridize to, and are therefore the complements of, the nucleic acid sequences (a) through (d), in the preceding paragraph.

Functional equivalents of the HGPRBMY2 include naturally occurring HGPRBMY2 present in other species, *i.e.*, orthologs, and mutant HGPRBMY2 whether naturally occurring or engineered. The invention also includes degenerate variants of sequences (a) through (d), *supra*. The invention also includes nucleic acid molecules, preferably DNA molecules, that hybridize to, and are therefore the complements of, the nucleic acid sequences (a) through (d), in the preceding paragraph.

Hybridization conditions may be highly stringent or less highly stringent. In instances wherein the nucleic acid molecules are deoxyoligonucleotides (“oligos”), highly stringent conditions may refer, *e.g.*, to washing in 6x SSC/0.05% sodium pyrophosphate

at 37°C (for 14-base oligos), 48°C (for 17-base oligos), 55°C (for 20-base oligos), and 60°C (for 23-base oligos). These nucleic acid molecules may encode or act as HGPRBMY1 or HGPRBMY2 antisense molecules, useful, for example, in HGPRBMY1 or HGPRBMY2 gene regulation (for and/or as antisense primers in amplification reactions of HGPRBMY1 or HGPRBMY2 gene nucleic acid sequences).

The invention features nucleic acids that are similar to the HGPRBMY1 nucleic acid sequences of the invention. A nucleic acid that has a similar sequence refers to a nucleic acid that satisfies at least one of the following: (a) a nucleic acid having a sequence that is at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% or at least 99% identical to the nucleic acid sequence of a GPCR as described herein; (b) a nucleic acid as described herein of at least 100 nucleotides, or at least 125, 150, 200, 250, 300, 400, 500, 600, 700, 800, 900, 1000, 1100, 1250, 1350, 1500, 1650, 1750, 1850, 2000, 2150, 2250 or 2400 contiguous nucleotides in length; and (c) a nucleic acid that is at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% or at least 99% identical to the nucleic acid sequence encoding a GPCR polypeptide or peptide as described herein.

The invention features nucleic acids that are similar to the HGPRBMY2 nucleic acid sequences of the invention. A nucleic acid that has a similar sequence refers to a nucleic acid that satisfies at least one of the following: (a) a nucleic acid having a sequence that is at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% or at least 99% identical to the nucleic acid sequence of a GPCR as described herein; (b) a nucleic acid as described herein of at least 100 nucleotides, or at least 125, 150, 200, 250, 300, 400, 500, 600, 700, 800, 900, 1000, 1100, 1250, 1350, 1500, 1650, 1750, 1850, 2000, 2150, 2250 or 2400 contiguous nucleotides in length; and (c) a nucleic acid that is at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% or at least 99% identical to the nucleic acid sequence encoding a GPCR polypeptide or peptide as described herein.

The invention also features allelic variants, *i.e.*, functional equivalents of the 5 HGPRBMY1 or HGPRBMY2 nucleic acid sequence which are naturally occurring and appear in the same genetic locus.

Nucleic acids of HGPRBMY1 or HGPRBMY2 can also be used to identify species orthologs of the sequence, *e.g.*, in monkeys, mice, cats, dogs, cows, fruit flies, 10 zebrafish or other animals. The identification of orthologs of HGPRBMY1 or HGPRBMY2 in other species can be useful for developing animal model systems more closely related to humans for purposes of drug discovery. For example, expression 15 libraries of cDNAs synthesized from bone marrow mRNA derived from the organism of interest can be screened using labeled agonist derived from that species, *e.g.*, an alkaline phosphatase (AP)-agonist fusion polypeptide.

Sequences of the invention may be used as part of ribozyme and/or triple helix sequences, also useful for HGPRBMY1 gene regulation. Still further, such molecules 20 may be used as components of diagnostic methods whereby, for example, the presence of a particular HGPRBMY1 allele responsible for causing an immune disorder, such as immunodeficiency, may be detected.

Sequences of the invention may be used as part of ribozyme and/or triple helix sequences, also useful for HGPRBMY2 gene regulation. Still further, such molecules 25 may be used as components of diagnostic methods whereby, for example, the presence of a particular HGPRBMY2 allele responsible for causing a heart disorder, such as heart failure, may be detected.

In addition to the HGPRBMY1 nucleic acid sequences described above, full length HGPRBMY1 cDNA or gene sequences present in the same species and/or homologues of the HGPRBMY1 gene present in other species can be identified and 30 readily isolated, without undue experimentation, by molecular biological techniques well known in the art. The identification of homologues of HGPRBMY1 in related species can be useful for developing animal model systems more closely related to humans for purposes of drug discovery. For example, expression libraries of cDNAs synthesized from spleen or bone marrow mRNA derived from the organism of interest can be 35 screened using labeled agonist derived from that species, *e.g.*, an AP-agonist fusion polypeptide.

In addition to the HGPRBMY2 nucleic acid sequences described above, full 5 length HGPRBMY2 cDNA or gene sequences present in the same species and/or homologues of the HGPRBMY2 gene present in other species can be identified and readily isolated, without undue experimentation, by molecular biological techniques well known in the art. The identification of homologues of HGPRBMY2 in related species can be useful for developing animal model systems more closely related to humans for 10 purposes of drug discovery. For example, expression libraries of cDNAs synthesized from heart mRNA derived from the organism of interest can be screened using labeled agonist derived from that species, *e.g.*, an AP-agonist fusion polypeptide.

Alternatively, such cDNA libraries, or genomic DNA libraries derived from the 15 organism of interest can be screened by hybridization using the nucleic acids described herein as hybridization or amplification probes. Furthermore, genes at other genetic loci within the genome that encode proteins which have extensive homology to one or more domains of the HGPRBMY1 or HGPRBMY2 gene product can also be identified via 20 similar techniques. In the case of cDNA libraries, such screening techniques can identify clones derived from alternatively spliced transcripts in the same or different species.

Screening can be by filter hybridization, using duplicate filters. The labeled probe 25 can contain at least 15-30 base pairs of the HGPRBMY1 or HGPRBMY2 nucleic acid sequence, as shown in Figure 1 or Figure 6. The hybridization washing conditions used should be of a lower stringency when the cDNA library is derived from an organism 30 different from the type of organism from which the labeled sequence was derived. With respect to the cloning of a human HGPRBMY1 or HGPRBMY2 homolog, using murine HGPRBMY1 or HGPRBMY2 probes, for example, hybridization can, for example, be performed at 65°C overnight in Church's buffer (7% SDS, 250 mM NaHPO<sub>4</sub>, 2 mM EDTA, 1% BSA). Washes can be done with 2x SSC, 0.1% SDS at 65°C and then at 0.1x 35 SSC, 0.1% SDS at 65°C.

Low stringency conditions are well known to those of skill in the art, and will vary predictably depending on the specific organisms from which the library and the labeled sequences are derived. For guidance regarding such conditions see, for example, 35 Sambrook et al., 1989, Molecular Cloning, A Laboratory Manual, Cold Springs Harbor Press, N.Y.; and Ausubel et al., 1989, Current Protocols in Molecular Biology, Green Publishing Associates and Wiley Interscience, N.Y.

Alternatively, the labeled HGPRBMY1 or HGPRBMY2 nucleic acid probe may  
5 be used to screen a genomic library derived from the organism of interest, again, using  
appropriately stringent conditions. The identification and characterization of human  
genomic clones is helpful for designing diagnostic tests and clinical protocols for treating  
cardiovascular disorders in human patients. For example, sequences derived from regions  
10 adjacent to the intron/exon boundaries of the human gene can be used to design primers  
for use in amplification assays to detect mutations within the exons, introns, splice sites  
(*e.g.* splice acceptor and/or donor sites), etc., that can be used in diagnostics.

Further, an HGPRBMY1 or HGPRBMY2 gene homologue may be isolated from  
nucleic acid of the organism of interest by performing PCR using two degenerate  
15 oligonucleotide primer pools designed on the basis of amino acid sequences within the  
HGPRBMY1 or HGPRBMY2 gene product disclosed herein. The template for the  
reaction may be cDNA obtained by reverse transcription of mRNA prepared from, for  
example, human or non-human cell lines or tissue, such as bone marrow, known or  
suspected to express an HGPRBMY1 or HGPRBMY2 gene allele.

20 The PCR product may be subcloned and sequenced to ensure that the amplified  
sequences represent the sequences of an HGPRBMY1 or HGPRBMY2 gene. The PCR  
fragment may then be used to isolate a full length cDNA clone by a variety of methods.  
For example, the amplified fragment may be labeled and used to screen a cDNA library,  
25 such as a bacteriophage cDNA library. Alternatively, the labeled fragment may be used  
to isolate genomic clones via the screening of a genomic library.

PCR technology may also be utilized to isolate full length cDNA sequences. For  
example, RNA may be isolated, following standard procedures, from an appropriate  
cellular or tissue source (*i.e.*, one known, or suspected, to express the HGPRBMY1 gene,  
30 such as, for example, spleen or bone marrow). A reverse transcription reaction may be  
performed on the RNA using an oligonucleotide primer specific for the most 5' end of the  
amplified fragment for the priming of first strand synthesis. The resulting RNA/DNA  
hybrid may then be "tailed" with guanines using a standard terminal transferase reaction,  
35 the hybrid may be digested with RNAase H, and second strand synthesis may then be  
primed with a poly-C primer. Thus, cDNA sequences upstream of the amplified fragment  
may easily be isolated. (For a review of cloning strategies which may be used, see *e.g.*,  
Sambrook et al., 1989, *supra*).

PCR technology may also be utilized to isolate full length cDNA sequences. For 5 example, RNA may be isolated, following standard procedures, from an appropriate cellular or tissue source (*i.e.*, one known, or suspected, to express the HGPRBMY2 gene, such as, for example, heart tissues). A reverse transcription reaction may be performed on the RNA using an oligonucleotide primer specific for the most 5' end of the amplified 10 fragment for the priming of first strand synthesis. The resulting RNA/DNA hybrid may then be "tailed" with guanines using a standard terminal transferase reaction, the hybrid may be digested with RNAase H, and second strand synthesis may then be primed with a poly-C primer. Thus, cDNA sequences upstream of the amplified fragment may easily 15 be isolated. (For a review of cloning strategies which may be used, see *e.g.*, Sambrook et al., 1989, *supra*.)

The HGPRBMY1 gene sequences may additionally be used to isolate mutant 20 HGPRBMY1 gene alleles. Such mutant alleles may be isolated from individuals either known or proposed to have a genotype which contributes to the symptoms of immune disorders. Mutant alleles and mutant allele products may then be utilized in the 25 therapeutic and diagnostic systems described below. Additionally, such HGPRBMY1 gene sequences can be used to detect HGPRBMY1 gene regulatory (*e.g.*, promoter or promoter/enhancer) defects which can affect immune function.

The HGPRBMY2 gene sequences may additionally be used to isolate mutant 25 HGPRBMY2 gene alleles. Such mutant alleles may be isolated from individuals either known or proposed to have a genotype which contributes to the symptoms of cardiovascular disorders. Mutant alleles and mutant allele products may then be utilized in the therapeutic and diagnostic systems described below. Additionally, such 30 HGPRBMY2 gene sequences can be used to detect HGPRBMY2 gene regulatory (*e.g.*, promoter or promotor/enhancer) defects which can affect cardiovascular function.

A cDNA of a mutant HGPRBMY1 or HGPRBMY2 gene may be isolated, for example, by using PCR, a technique which is well known to those of skill in the art. In this case, the first cDNA strand may be synthesized by hybridizing an oligo-dT 35 oligonucleotide to mRNA isolated from tissue known or suspected to be expressed in an individual putatively carrying the mutant HGPRBMY1 or HGPRBMY2 allele, and by extending the new strand with reverse transcriptase. The second strand of the cDNA is then synthesized using an oligonucleotide that hybridizes specifically to the 5' end of the

normal gene. Using these two primers, the product is then amplified via PCR, cloned into 5 a suitable vector, and subjected to DNA sequence analysis through methods well known to those of skill in the art. By comparing the DNA sequence of the mutant HGPRBMY1 or HGPRBMY2 allele to that of the normal HGPRBMY1 or HGPRBMY2 allele, the mutation(s) responsible for the loss or alteration of function of the mutant HGPRBMY1 or HGPRBMY2 gene product can be ascertained.

10 Alternatively, a genomic library can be constructed using DNA obtained from an individual suspected of or known to carry the mutant HGPRBMY1 or HGPRBMY2 allele, or a cDNA library can be constructed using RNA from a tissue known, or suspected, to express the mutant HGPRBMY1 or HGPRBMY2 allele. The normal 15 HGPRBMY1 or HGPRBMY2 gene or any suitable fragment thereof may then be labeled and used as a probe to identify the corresponding mutant HGPRBMY1 or HGPRBMY2 allele in such libraries. Clones containing the mutant HGPRBMY1 or HGPRBMY2 gene sequences may then be purified and subjected to sequence analysis according to methods well known to those of skill in the art.

20 Additionally, an expression library can be constructed utilizing cDNA synthesized from, for example, RNA isolated from a tissue known, or suspected, to express a mutant HGPRBMY1 or HGPRBMY2 allele in an individual suspected of or known to carry such a mutant allele. In this manner, gene products made by the putatively 25 mutant tissue may be expressed and screened using standard antibody screening techniques in conjunction with antibodies raised against the normal HGPRBMY1 or HGPRBMY2 gene product, as described, below, in Section 5.3. (For screening techniques, see, for example, Harlow, E. and Lane, eds., 1988, "Antibodies: A Laboratory Manual", Cold Spring Harbor Press, Cold Spring Harbor.) Additionally, screening can 30 be accomplished by screening with labeled agonist or antagonist fusion polypeptides, such as, for example, AP-GPCR or GPCR-AP fusion polypeptides. In cases where an HGPRBMY1 or HGPRBMY2 mutation results in an expressed gene product with altered function (e.g., as a result of a missense or a frameshift mutation), a polyclonal set of 35 antibodies to HGPRBMY1 or HGPRBMY2 are likely to cross-react with the mutant HGPRBMY1 or HGPRBMY2 gene product. Library clones detected via their reaction with such labeled antibodies can be purified and subjected to sequence analysis according to methods well known to those of skill in the art.

HGPRBMY1 or HGPRBMY2 nucleic acids can also be utilized for chromosomal  
5 mapping, or as chromosomal markers, *e.g.*, in radiation hybrid mapping.

The invention also features identifying detecting or diagnosing cells or tissues which express a mRNA or HGPRBMY1 or HGPRBMY2. The invention also features nucleic acid sequences that encode mutant HGPRBMY1 or HGPRBMY2 polypeptides, peptides of the HGPRBMY1 or HGPRBMY2, truncated HGPRBMY1 or HGPRBMY2, 10 and HGPRBMY1 or HGPRBMY2 fusion polypeptides. These include, but are not limited to nucleic acid sequences encoding mutant HGPRBMY1 or HGPRBMY2 described in section 5.2 infra; polypeptides or peptides corresponding to the ECD, TM and/or CD domains of the HGPRBMY1 or HGPRBMY2 or portions of these domains; truncated 15 HGPRBMY1 or HGPRBMY2 in which one or two of the domains are deleted, *e.g.*, a soluble HGPRBMY1 or HGPRBMY2 lacking the TM or both the TM and CD regions, or a truncated, nonfunctional HGPRBMY1 or HGPRBMY2 lacking all or a portion of the CD region. Nucleotides encoding fusion polypeptides may include by are not limited to full length HGPRBMY1 or HGPRBMY2, HGPRBMY1 or HGPRBMY2 peptides, or 20 HGPRBMY1 or HGPRBMY2 polypeptides or peptides fused to an unrelated polypeptide or peptide, such as for example, a transmembrane sequence, which anchors the HGPRBMY1 or HGPRBMY2 ECD to the cell membrane; an Ig-Fc domain which increases the stability and half life of the resulting fusion polypeptide (*e.g.*, HGPRBMY1 or HGPRBMY2-Ig) in the bloodstream; or an enzyme, fluorescent polypeptide, 25 luminescent polypeptide which can be used as a marker.

The invention also encompasses (a) DNA vectors that contain any of the foregoing HGPRBMY1 or HGPRBMY2 coding sequences and/or their complements (*i.e.*, antisense); (b) DNA expression vectors that contain any of the foregoing 30 HGPRBMY1 or HGPRBMY2 coding sequences operatively associated with a regulatory element that directs the expression of the coding sequences; and (c) genetically engineered host cells that contain any of the foregoing HGPRBMY1 or HGPRBMY2 coding sequences operatively associated with a regulatory element that directs the expression of the coding sequences in the host cell.

35 As used herein, regulatory elements include but are not limited to inducible and non-inducible promoters, enhancers, operators and other elements known to those skilled in the art that drive and regulate expression. Such regulatory elements include but are not

limited to the cytomegalovirus hCMV immediate early gene, the early or late promoters of SV40 adenovirus, the lac system, the trp system, the TAC system, the TRC system, the major operator and promoter regions of phage A, the control regions of fd coat polypeptide, the promoter for 3-phosphoglycerate kinase, the promoters of acid phosphatase, and the promoters of the yeast  $\alpha$ -mating factors.

These expression and cloning methods include, for example, *in vitro* recombinant DNA techniques, synthetic techniques, and *in vivo* genetic recombination. (For example, Sambrook et al., 1989, *supra*, and Ausubel et al., 1989, *supra*). Alternatively, RNA capable of encoding HGPRBMY1 nucleic acid sequences may be chemically synthesized using, for example, synthesizers. See, for example, the techniques described in "Oligonucleotide Synthesis", 1984, Gait, M. J. ed., IRL Press, Oxford, which is incorporated by reference herein in its entirety.

As referenced elsewhere herein, characterization of the HGPRBMY1 polypeptide of the present invention led to the determination that it is involved in the modulation of the cyclin p27 protein, in addition to, the apoptosis regulatory protein I $\kappa$ B, either directly or indirectly.

In preferred embodiments, HGPRBMY1 polynucleotides and polypeptides, including fragments thereof, are useful for treating, diagnosing, and/or ameliorating cell cycle defects, disorders related to aberrant phosphorylation, disorders related to aberrant signal transduction, proliferating disorders, and/or cancers.

In preferred embodiments, antagonists directed to HGPRBMY1 are useful for decreasing cellular proliferation, decreasing cellular proliferation in rapidly proliferating cells, increasing the number of cells in the G1 phase of the cell cycle, and decreasing the number of cells that progress to the S phase of the cell cycle.

Moreover, agonists directed against HGPRBMY1 are useful for increasing cellular proliferation, increasing cellular proliferation in rapidly proliferating cells, decreasing the number of cells in the G1 phase of the cell cycle, and increasing the number of cells that progress to the S phase of the cell cycle. Such agonists would be particularly useful for transforming normal cells into immortalized cell lines, stimulating hematopoietic cells to grow and divide, increasing recovery rates of cancer patients that have undergone chemotherapy or other therapeutic regimen, by boosting their immune responses, etc.

In preferred embodiments, HGPRBMY1 polynucleotides and polypeptides, 5 including fragments thereof, are useful for treating, diagnosing, and/or ameliorating proliferative disorders, cancers, ischemia-reperfusion injury, heart failure, immuno compromised conditions, HIV infection, and renal diseases.

Moreover, HGPRBMY1 polynucleotides and polypeptides, including fragments thereof, are useful for increasing NF- $\kappa$ B activity, decreasing apoptotic events, and/or 10 decreasing I $\square$ B $\square$  expression or activity levels.

In preferred embodiments, antagonists directed against HGPRBMY1 are useful for treating, diagnosing, and/or ameliorating autoimmune disorders, disorders related to hyper immune activity, inflammatory conditions, disorders related to aberrant acute phase 15 responses, hypercongenital conditions, birth defects, necrotic lesions, wounds, organ transplant rejection, conditions related to organ transplant rejection, disorders related to aberrant signal transduction, proliferating disorders, cancers, HIV, and HIV propagation in cells infected with other viruses.

Moreover, antagonists directed against HGPRBMY1 are useful for decreasing 20 NF- $\kappa$ B activity, increasing apoptotic events, and/or increasing I $\square$ B $\square$  expression or activity levels.

In preferred embodiments, agonists directed against HGPRBMY1 are useful for treating, diagnosing, and/or ameliorating autoimmune disorders, disorders related to hyper 25 immune activity, hypercongenital conditions, birth defects, necrotic lesions, wounds, disorders related to aberrant signal transduction, immuno compromised conditions, HIV infection, proliferating disorders, Alzheimer's, and/or cancers.

Moreover, agonists directed against HGPRBMY1 are useful for increasing NF- $\kappa$ B activity, decreasing apoptotic events, and/or decreasing I $\square$ B $\square$  expression or activity 30 levels.

## 5.2. HGPRBMY1 and HGPRBMY2 Polypeptides

The term "peptides" as used herein is meant to comprise a small number of amino acids connected by peptide bonds. The term "polypeptide" generally refers to longer 35 chains of amino acids but does not refer to a specific length, thus as used herein, polypeptides include proteins (a term usually reserved for a functional unit which may consist of either a single polypeptide or several polypeptides).

The invention features polypeptides and/or peptides that are similar to the sequence of HGPRBMY1. A polypeptide or peptide that has a similar amino acid sequence refers to a polypeptide or peptide sequence that satisfies at least one of the following: (a) a polypeptide having an amino acid sequence that is at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% or at least 99% identical to the amino acid sequence of a GPCR polypeptide or peptide as described herein; (b) a polypeptide or peptide encoded by a nucleic acid sequence that hybridizes under stringent conditions to a nucleic acid sequence encoding a GPCR as described herein of at least 20 amino acid residues, at least 25, at least 40, at least 50, at least 60, at least 70, at least 80, at least 90, at least 100, at least 125, at least 150, at least 200, at least 225, at least 250, at least 275, at least 300 or at least 350 amino acids; and (c) a polypeptide encoded by a nucleic acid sequence that is at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% or at least 99% identical to the nucleic acid sequence encoding a GPCR polypeptide or peptide as described herein.

The invention features polypeptides and/or peptides that are similar to the sequence of HGPRBMY2. A polypeptide or peptide that has a similar amino acid sequence refers to a polypeptide or peptide sequence that satisfies at least one of the following: (a) a polypeptide having an amino acid sequence that is at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% or at least 99% identical to the amino acid sequence of a GPCR polypeptide or peptide as described herein; (b) a polypeptide or peptide encoded by a nucleic acid sequence that hybridizes under stringent conditions to a nucleic acid sequence encoding a GPCR as described herein of at least 20 amino acid residues, at least 25, at least 40, at least 50, at least 60, at least 70, at least 80, at least 90, at least 100, at least 125, at least 150, at least 200, at least 225, at least 250, at least 275, at least 300 or at least 350 amino acids; and (c) a polypeptide encoded by a nucleic acid sequence that is at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% or at least 99% identical to the nucleic acid sequence encoding a GPCR polypeptide or peptide as described herein.

A polypeptide with similar structure and/or function to a GPCR polypeptide or  
5 as described herein refers to a polypeptide that has a similar secondary, tertiary or  
quaternary structure of a GPCR polypeptide, *e.g.*, a protein or a fusion protein, as  
described herein. The structure of a polypeptide can be determined by methods known to  
those skilled in the art, including but not limited to, X-ray crystallography, nuclear  
magnetic resonance, and crystallographic electron microscopy.

10 HGPRBMY1 polypeptides and peptides, mutations, truncations and/or  
HGPRBMY1 fusion polypeptides of any of the foregoing can be used for, but not limited  
to, the generation of antibodies, as reagents in diagnostic assays, the identification of  
other cellular gene products involved in the regulation of immune function, as reagents  
15 in assays for screening for compounds that can be used in the treatment of immune  
disorders, and as pharmaceutical reagents useful in the treatment of immune disorders  
related to the HGPRBMY1.

In preferred embodiments, the following N-terminal HGPRBMY1 deletion  
polypeptides are encompassed by the present invention: M1-F359, Q2-F359, V3-F359,  
20 P4-F359, N5-F359, S6-F359, T7-F359, G8-F359, P9-F359, D10-F359, N11-F359, A12-  
F359, T13-F359, L14-F359, Q15-F359, M16-F359, L17-F359, R18-F359, N19-F359,  
P20-F359, A21-F359, I22-F359, A23-F359, V24-F359, A25-F359, L26-F359, P27-F359,  
V28-F359, V29-F359, Y30-F359, S31-F359, L32-F359, V33-F359, A34-F359, A35-  
25 F359, V36-F359, S37-F359, I38-F359, P39-F359, G40-F359, N41-F359, L42-F359, F43-  
F359, S44-F359, L45-F359, W46-F359, V47-F359, L48-F359, C49-F359, R50-F359,  
R51-F359, M52-F359, G53-F359, P54-F359, R55-F359, S56-F359, P57-F359, S58-  
F359, V59-F359, I60-F359, F61-F359, M62-F359, I63-F359, N64-F359, L65-F359, S66-  
F359, V67-F359, T68-F359, D69-F359, L70-F359, M71-F359, L72-F359, A73-F359,  
30 S74-F359, V75-F359, L76-F359, P77-F359, F78-F359, Q79-F359, I80-F359, Y81-F359,  
Y82-F359, H83-F359, C84-F359, N85-F359, R86-F359, H87-F359, H88-F359, W89-  
F359, V90-F359, F91-F359, G92-F359, V93-F359, L94-F359, L95-F359, C96-F359,  
N97-F359, V98-F359, V99-F359, T100-F359, V101-F359, A102-F359, F103-F359,  
35 Y104-F359, A105-F359, N106-F359, M107-F359, Y108-F359, S109-F359, S110-F359,  
I111-F359, L112-F359, T113-F359, M114-F359, T115-F359, C116-F359, I117-F359,  
S118-F359, V119-F359, E120-F359, R121-F359, F122-F359, L123-F359, G124-F359,  
V125-F359, L126-F359, Y127-F359, P128-F359, L129-F359, S130-F359, S131-F359,

K132-F359, R133-F359, W134-F359, R135-F359, R136-F359, R137-F359, R138-F359,  
5 Y139-F359, A140-F359, V141-F359, A142-F359, A143-F359, C144-F359, A145-F359,  
G146-F359, T147-F359, W148-F359, L149-F359, L150-F359, L151-F359, L152-F359,  
T153-F359, A154-F359, L155-F359, S156-F359, P157-F359, L158-F359, A159-F359,  
R160-F359, T161-F359, D162-F359, L163-F359, T164-F359, Y165-F359, P166-F359,  
10 V167-F359, H168-F359, A169-F359, L170-F359, G171-F359, I172-F359, I173-F359,  
T174-F359, C175-F359, F176-F359, D177-F359, V178-F359, L179-F359, K180-F359,  
W181-F359, T182-F359, M183-F359, L184-F359, P185-F359, S186-F359, V187-F359,  
A188-F359, M189-F359, W190-F359, A191-F359, V192-F359, F193-F359, L194-F359,  
F195-F359, T196-F359, I197-F359, F198-F359, I199-F359, L200-F359, L201-F359,  
15 F202-F359, L203-F359, I204-F359, P205-F359, F206-F359, V207-F359, I208-F359,  
T209-F359, V210-F359, A211-F359, C212-F359, Y213-F359, T214-F359, A215-F359,  
T216-F359, I217-F359, L218-F359, K219-F359, L220-F359, L221-F359, R222-F359,  
T223-F359, E224-F359, E225-F359, A226-F359, H227-F359, G228-F359, R229-F359,  
E230-F359, Q231-F359, R232-F359, R233-F359, R234-F359, A235-F359, V236-F359,  
20 G237-F359, L238-F359, A239-F359, A240-F359, V241-F359, V242-F359, L243-F359,  
L244-F359, A245-F359, F246-F359, V247-F359, T248-F359, C249-F359, F250-F359,  
A251-F359, P252-F359, N253-F359, N254-F359, F255-F359, V256-F359, L257-F359,  
L258-F359, A259-F359, H260-F359, I261-F359, V262-F359, S263-F359, R264-F359,  
25 L265-F359, F266-F359, Y267-F359, G268-F359, K269-F359, S270-F359, Y271-F359,  
Y272-F359, H273-F359, V274-F359, Y275-F359, K276-F359, L277-F359, T278-F359,  
L279-F359, C280-F359, L281-F359, S282-F359, C283-F359, L284-F359, N285-F359,  
N286-F359, C287-F359, L288-F359, D289-F359, P290-F359, F291-F359, V292-F359,  
Y293-F359, Y294-F359, F295-F359, A296-F359, S297-F359, R298-F359, E299-F359,  
30 F300-F359, Q301-F359, L302-F359, R303-F359, L304-F359, R305-F359, E306-F359,  
Y307-F359, L308-F359, G309-F359, C310-F359, R311-F359, R312-F359, V313-F359,  
P314-F359, R315-F359, D316-F359, T317-F359, L318-F359, D319-F359, T320-F359,  
R321-F359, R322-F359, E323-F359, S324-F359, L325-F359, F326-F359, S327-F359,  
35 A328-F359, R329-F359, T330-F359, T331-F359, S332-F359, V333-F359, R334-F359,  
S335-F359, E336-F359, A337-F359, G338-F359, A339-F359, H340-F359, P341-F359,  
E342-F359, G343-F359, M344-F359, E345-F359, G346-F359, A347-F359, T348-F359,  
R349-F359, P350-F359, G351-F359, L352-F359, and/or Q353-F359 of SEQ ID NO:2.

5 Polynucleotide sequences encoding these polypeptides are also provided. The present invention also encompasses the use of these N-terminal HGPRBMY1 deletion polypeptides as immunogenic and/or antigenic epitopes as described elsewhere herein.

In preferred embodiments, the following C-terminal HGPRBMY1 deletion polypeptides are encompassed by the present invention: M1-F359, M1-V358, M1-S357, 10 M1-E356, M1-Q355, M1-R354, M1-Q353, M1-L352, M1-G351, M1-P350, M1-R349, M1-T348, M1-A347, M1-G346, M1-E345, M1-M344, M1-G343, M1-E342, M1-P341, M1-H340, M1-A339, M1-G338, M1-A337, M1-E336, M1-S335, M1-R334, M1-V333, 15 M1-S332, M1-T331, M1-T330, M1-R329, M1-A328, M1-S327, M1-F326, M1-L325, M1-S324, M1-E323, M1-R322, M1-R321, M1-T320, M1-D319, M1-L318, M1-T317, 20 M1-D316, M1-R315, M1-P314, M1-V313, M1-R312, M1-R311, M1-C310, M1-G309, M1-L308, M1-Y307, M1-E306, M1-R305, M1-L304, M1-R303, M1-L302, M1-Q301, M1-F300, M1-E299, M1-R298, M1-S297, M1-A296, M1-F295, M1-Y294, M1-Y293, 25 M1-V292, M1-F291, M1-P290, M1-D289, M1-L288, M1-C287, M1-N286, M1-N285, M1-L284, M1-C283, M1-S282, M1-L281, M1-C280, M1-L279, M1-T278, M1-L277, 30 M1-K276, M1-Y275, M1-V274, M1-H273, M1-Y272, M1-Y271, M1-S270, M1-K269, M1-G268, M1-Y267, M1-F266, M1-L265, M1-R264, M1-S263, M1-V262, M1-I261, M1-H260, M1-A259, M1-L258, M1-L257, M1-V256, M1-F255, M1-N254, M1-N253, 35 M1-P252, M1-A251, M1-F250, M1-C249, M1-T248, M1-V247, M1-F246, M1-A245, M1-L244, M1-L243, M1-V242, M1-V241, M1-A240, M1-A239, M1-L238, M1-G237, M1-V236, M1-A235, M1-R234, M1-R233, M1-R232, M1-Q231, M1-E230, M1-R229, M1-G228, M1-H227, M1-A226, M1-E225, M1-E224, M1-T223, M1-R222, M1-L221, M1-L220, M1-K219, M1-L218, M1-I217, M1-T216, M1-A215, M1-T214, M1-Y213, M1-C212, M1-A211, M1-V210, M1-T209, M1-I208, M1-V207, M1-F206, M1-P205, 40 M1-I204, M1-L203, M1-F202, M1-L201, M1-L200, M1-I199, M1-F198, M1-I197, M1-T196, M1-F195, M1-L194, M1-F193, M1-V192, M1-A191, M1-W190, M1-M189, M1-A188, M1-V187, M1-S186, M1-P185, M1-L184, M1-M183, M1-T182, M1-W181, M1-K180, M1-L179, M1-V178, M1-D177, M1-F176, M1-C175, M1-T174, M1-I173, M1-I172, M1-G171, M1-L170, M1-A169, M1-H168, M1-V167, M1-P166, M1-Y165, M1-T164, M1-L163, M1-D162, M1-T161, M1-R160, M1-A159, M1-L158, M1-P157, M1-S156, M1-L155, M1-A154, M1-T153, M1-L152, M1-L151, M1-L150, M1-L149, M1-W148, M1-T147, M1-G146, M1-A145, M1-C144, M1-A143, M1-A142, M1-V141, M1- 45

5 A140, M1-Y139, M1-R138, M1-R137, M1-R136, M1-R135, M1-W134, M1-R133, M1-  
K132, M1-S131, M1-S130, M1-L129, M1-P128, M1-Y127, M1-L126, M1-V125, M1-  
G124, M1-L123, M1-F122, M1-R121, M1-E120, M1-V119, M1-S118, M1-I117, M1-  
C116, M1-T115, M1-M114, M1-T113, M1-L112, M1-I111, M1-S110, M1-S109, M1-  
Y108, M1-M107, M1-N106, M1-A105, M1-Y104, M1-F103, M1-A102, M1-V101, M1-  
T100, M1-V99, M1-V98, M1-N97, M1-C96, M1-L95, M1-L94, M1-V93, M1-G92, M1-  
10 F91, M1-V90, M1-W89, M1-H88, M1-H87, M1-R86, M1-N85, M1-C84, M1-H83, M1-  
Y82, M1-Y81, M1-I80, M1-Q79, M1-F78, M1-P77, M1-L76, M1-V75, M1-S74, M1-  
A73, M1-L72, M1-M71, M1-L70, M1-D69, M1-T68, M1-V67, M1-S66, M1-L65, M1-  
N64, M1-I63, M1-M62, M1-F61, M1-I60, M1-V59, M1-S58, M1-P57, M1-S56, M1-  
15 R55, M1-P54, M1-G53, M1-M52, M1-R51, M1-R50, M1-C49, M1-L48, M1-V47, M1-  
W46, M1-L45, M1-S44, M1-F43, M1-L42, M1-N41, M1-G40, M1-P39, M1-I38, M1-  
S37, M1-V36, M1-A35, M1-A34, M1-V33, M1-L32, M1-S31, M1-Y30, M1-V29, M1-  
V28, M1-P27, M1-L26, M1-A25, M1-V24, M1-A23, M1-I22, M1-A21, M1-P20, M1-  
N19, M1-R18, M1-L17, M1-M16, M1-Q15, M1-L14, M1-T13, M1-A12, M1-N11, M1-  
20 D10, M1-P9, M1-G8, and/or M1-T7 of SEQ ID NO:2. Polynucleotide sequences  
encoding these polypeptides are also provided. The present invention also encompasses  
the use of these C-terminal HGPRBMY1 deletion polypeptides as immunogenic and/or  
antigenic epitopes as described elsewhere herein.

25 HGPRBMY2 polypeptides and peptides, mutated, truncated or deleted forms of  
the HGPRBMY2 and/or HGPRBMY2 fusion polypeptides can be prepared for a variety  
of uses, including but not limited to the generation of antibodies, as reagents in diagnostic  
assays, the identification of other cellular gene products involved in the regulation of  
cardiovascular, as reagents in assays for screening for compounds that can be used in the  
30 treatment of cardiovascular disorders, and as pharmaceutical reagents useful in the  
treatment of cardiovascular disorders related to the HGPRBMY2.

35 The deduced amino acid sequence encoded by the open reading frame of  
HGPRBMY2 is 431 amino acids (SEQ ID NO:14) and is shown in Figure 7. The  
extracellular domains ("ECD") of HGPRBMY2 extend from about amino acid residues  
1 to about 45, about 105 to about 119, about 182 to about 212, and about 293 to about  
311 of SEQ ID NO:14; the transmembrane domains of HGPRBMY2 extend from about  
amino acid residues 46 to about 69, about 82 to about 104, about 119 to about 141, about

162 to about 181, about 213 to about 233, about 272 to about 292, and about 312 to about  
5 335 of SEQ ID NO:14; and the cytoplasmic domains of HGPRBMY2 extend from about  
amino acid residue 69 to about 81, about 142 to about 161, about 234 to about 271, and  
about 336 to about 431 of SEQ ID NO:14.

In preferred embodiments, the following N-terminal HGPRBMY2 deletion  
10 polypeptides are encompassed by the present invention: M1-H431, Q2-H431, A3-H431,  
L4-H431, N5-H431, I6-H431, T7-H431, P8-H431, E9-H431, Q10-H431, F11-H431,  
S12-H431, R13-H431, L14-H431, L15-H431, R16-H431, D17-H431, H18-H431, N19-  
H431, L20-H431, T21-H431, R22-H431, E23-H431, Q24-H431, F25-H431, I26-H431,  
A27-H431, L28-H431, Y29-H431, R30-H431, L31-H431, R32-H431, P33-H431, L34-  
15 H431, V35-H431, Y36-H431, T37-H431, P38-H431, E39-H431, L40-H431, P41-H431,  
G42-H431, R43-H431, A44-H431, K45-H431, L46-H431, A47-H431, L48-H431, V49-  
H431, L50-H431, T51-H431, G52-H431, V53-H431, L54-H431, I55-H431, F56-H431,  
A57-H431, L58-H431, A59-H431, L60-H431, F61-H431, G62-H431, N63-H431, A64-  
H431, L65-H431, V66-H431, F67-H431, Y68-H431, V69-H431, V70-H431, T71-H431,  
20 R72-H431, S73-H431, K74-H431, A75-H431, M76-H431, R77-H431, T78-H431, V79-  
H431, T80-H431, N81-H431, I82-H431, F83-H431, I84-H431, C85-H431, S86-H431,  
L87-H431, A88-H431, L89-H431, S90-H431, D91-H431, L92-H431, L93-H431, I94-  
H431, T95-H431, F96-H431, F97-H431, C98-H431, I99-H431, P100-H431, V101-H431,  
25 T102-H431, M103-H431, L104-H431, Q105-H431, N106-H431, I107-H431, S108-  
H431, D109-H431, N110-H431, W111-H431, L112-H431, G113-H431, G114-H431,  
A115-H431, F116-H431, I117-H431, C118-H431, K119-H431, M120-H431, V121-  
H431, P122-H431, F123-H431, V124-H431, Q125-H431, S126-H431, T127-H431,  
A128-H431, V129-H431, V130-H431, T131-H431, E132-H431, I133-H431, L134-H431,  
30 T135-H431, M136-H431, T137-H431, C138-H431, I139-H431, A140-H431, V141-  
H431, E142-H431, R143-H431, H144-H431, Q145-H431, G146-H431, L147-H431,  
V148-H431, H149-H431, P150-H431, F151-H431, K152-H431, M153-H431, K154-  
H431, W155-H431, Q156-H431, Y157-H431, T158-H431, N159-H431, R160-H431,  
35 R161-H431, A162-H431, F163-H431, T164-H431, M165-H431, L166-H431, G167-  
H431, V168-H431, V169-H431, W170-H431, L171-H431, V172-H431, A173-H431,  
V174-H431, I175-H431, V176-H431, G177-H431, S178-H431, P179-H431, M180-  
H431, W181-H431, H182-H431, V183-H431, Q184-H431, Q185-H431, L186-H431,

5 E187-H431, I188-H431, K189-H431, Y190-H431, D191-H431, F192-H431, L193-H431,  
Y194-H431, E195-H431, K196-H431, E197-H431, H198-H431, I199-H431, C200-  
H431, C201-H431, L202-H431, E203-H431, E204-H431, W205-H431, T206-H431,  
S207-H431, P208-H431, V209-H431, H210-H431, Q211-H431, K212-H431, I213-H431,  
Y214-H431, T215-H431, T216-H431, F217-H431, I218-H431, L219-H431, V220-H431,  
I221-H431, L222-H431, F223-H431, L224-H431, L225-H431, P226-H431, L227-H431,  
10 M228-H431, V229-H431, M230-H431, L231-H431, I232-H431, L233-H431, Y234-  
H431, S235-H431, K236-H431, I237-H431, G238-H431, Y239-H431, E240-H431,  
L241-H431, W242-H431, I243-H431, K244-H431, K245-H431, R246-H431, V247-  
H431, G248-H431, D249-H431, G250-H431, S251-H431, V252-H431, L253-H431,  
15 R254-H431, T255-H431, I256-H431, H257-H431, G258-H431, K259-H431, E260-  
H431, M261-H431, S262-H431, K263-H431, I264-H431, A265-H431, R266-H431,  
K267-H431, K268-H431, K269-H431, R270-H431, A271-H431, V272-H431, I273-  
H431, M274-H431, M275-H431, V276-H431, T277-H431, V278-H431, V279-H431,  
20 A280-H431, L281-H431, F282-H431, A283-H431, V284-H431, C285-H431, W286-  
H431, A287-H431, P288-H431, F289-H431, H290-H431, V291-H431, V292-H431,  
H293-H431, M294-H431, M295-H431, I296-H431, E297-H431, Y298-H431, S299-  
H431, N300-H431, F301-H431, E302-H431, K303-H431, E304-H431, Y305-H431,  
D306-H431, D307-H431, V308-H431, T309-H431, I310-H431, K311-H431, M312-  
25 H431, I313-H431, F314-H431, A315-H431, I316-H431, V317-H431, Q318-H431, I319-  
H431, I320-H431, G321-H431, F322-H431, S323-H431, N324-H431, S325-H431, I326-  
H431, C327-H431, N328-H431, P329-H431, I330-H431, V331-H431, Y332-H431,  
A333-H431, F334-H431, M335-H431, N336-H431, E337-H431, N338-H431, F339-  
H431, K340-H431, K341-H431, N342-H431, V343-H431, L344-H431, S345-H431,  
30 A346-H431, V347-H431, C348-H431, Y349-H431, C350-H431, I351-H431, V352-  
H431, N353-H431, K354-H431, T355-H431, F356-H431, S357-H431, P358-H431,  
A359-H431, Q360-H431, R361-H431, H362-H431, G363-H431, N364-H431, S365-  
H431, G366-H431, I367-H431, T368-H431, M369-H431, M370-H431, R371-H431,  
K372-H431, K373-H431, A374-H431, K375-H431, F376-H431, S377-H431, L378-  
35 H431, R379-H431, E380-H431, N381-H431, P382-H431, V383-H431, E384-H431,  
E385-H431, T386-H431, K387-H431, G388-H431, E389-H431, A390-H431, F391-  
H431, S392-H431, D393-H431, G394-H431, N395-H431, I396-H431, E397-H431,

V398-H431, K399-H431, L400-H431, C401-H431, E402-H431, Q403-H431, T404-  
5 H431, E405-H431, E406-H431, K407-H431, K408-H431, K409-H431, L410-H431,  
K411-H431, R412-H431, H413-H431, L414-H431, A415-H431, L416-H431, F417-  
H431, R418-H431, S419-H431, E420-H431, L421-H431, A422-H431, E423-H431,  
N424-H431, and/or S425-H431 of SEQ ID NO:14. Polynucleotide sequences encoding  
10 these polypeptides are also provided. The present invention also encompasses the use of  
these N-terminal HGPRBMY2 deletion polypeptides as immunogenic and/or antigenic  
epitopes as described elsewhere herein.

In preferred embodiments, the following C-terminal HGPRBMY2 deletion  
polypeptides are encompassed by the present invention: M1-H431, M1-G430, M1-S429,  
15 M1-D428, M1-L427, M1-P426, M1-S425, M1-N424, M1-E423, M1-A422, M1-L421,  
M1-E420, M1-S419, M1-R418, M1-F417, M1-L416, M1-A415, M1-L414, M1-H413,  
M1-R412, M1-K411, M1-L410, M1-K409, M1-K408, M1-K407, M1-E406, M1-E405,  
M1-T404, M1-Q403, M1-E402, M1-C401, M1-L400, M1-K399, M1-V398, M1-E397,  
M1-I396, M1-N395, M1-G394, M1-D393, M1-S392, M1-F391, M1-A390, M1-E389,  
20 M1-G388, M1-K387, M1-T386, M1-E385, M1-E384, M1-V383, M1-P382, M1-N381,  
M1-E380, M1-R379, M1-L378, M1-S377, M1-F376, M1-K375, M1-A374, M1-K373,  
M1-K372, M1-R371, M1-M370, M1-M369, M1-T368, M1-I367, M1-G366, M1-S365,  
M1-N364, M1-G363, M1-H362, M1-R361, M1-Q360, M1-A359, M1-P358, M1-S357,  
25 M1-F356, M1-T355, M1-K354, M1-N353, M1-V352, M1-I351, M1-C350, M1-Y349,  
M1-C348, M1-V347, M1-A346, M1-S345, M1-L344, M1-V343, M1-N342, M1-K341,  
M1-K340, M1-F339, M1-N338, M1-E337, M1-N336, M1-M335, M1-F334, M1-A333,  
M1-Y332, M1-V331, M1-I330, M1-P329, M1-N328, M1-C327, M1-I326, M1-S325,  
M1-N324, M1-S323, M1-F322, M1-G321, M1-I320, M1-I319, M1-Q318, M1-V317,  
30 M1-I316, M1-A315, M1-F314, M1-I313, M1-M312, M1-K311, M1-I310, M1-T309, M1-  
V308, M1-D307, M1-D306, M1-Y305, M1-E304, M1-K303, M1-E302, M1-F301, M1-  
N300, M1-S299, M1-Y298, M1-E297, M1-I296, M1-M295, M1-M294, M1-H293, M1-  
V292, M1-V291, M1-H290, M1-F289, M1-P288, M1-A287, M1-W286, M1-C285, M1-  
V284, M1-A283, M1-F282, M1-L281, M1-A280, M1-V279, M1-V278, M1-T277, M1-  
35 V276, M1-M275, M1-M274, M1-I273, M1-V272, M1-A271, M1-R270, M1-K269, M1-  
K268, M1-K267, M1-R266, M1-A265, M1-I264, M1-K263, M1-S262, M1-M261, M1-  
E260, M1-K259, M1-G258, M1-H257, M1-I256, M1-T255, M1-R254, M1-L253, M1-

V252, M1-S251, M1-G250, M1-D249, M1-G248, M1-V247, M1-R246, M1-K245, M1-  
5 K244, M1-I243, M1-W242, M1-L241, M1-E240, M1-Y239, M1-G238, M1-I237, M1-  
K236, M1-S235, M1-Y234, M1-L233, M1-I232, M1-L231, M1-M230, M1-V229, M1-  
M228, M1-L227, M1-P226, M1-L225, M1-L224, M1-F223, M1-L222, M1-I221, M1-  
V220, M1-L219, M1-I218, M1-F217, M1-T216, M1-T215, M1-Y214, M1-I213, M1-  
10 K212, M1-Q211, M1-H210, M1-V209, M1-P208, M1-S207, M1-T206, M1-W205, M1-  
E204, M1-E203, M1-L202, M1-C201, M1-C200, M1-I199, M1-H198, M1-E197, M1-  
K196, M1-E195, M1-Y194, M1-L193, M1-F192, M1-D191, M1-Y190, M1-K189, M1-  
I188, M1-E187, M1-L186, M1-Q185, M1-Q184, M1-V183, M1-H182, M1-W181, M1-  
M180, M1-P179, M1-S178, M1-G177, M1-V176, M1-I175, M1-V174, M1-A173, M1-  
15 V172, M1-L171, M1-W170, M1-V169, M1-V168, M1-G167, M1-L166, M1-M165, M1-  
T164, M1-F163, M1-A162, M1-R161, M1-R160, M1-N159, M1-T158, M1-Y157, M1-  
Q156, M1-W155, M1-K154, M1-M153, M1-K152, M1-F151, M1-P150, M1-H149, M1-  
V148, M1-L147, M1-G146, M1-Q145, M1-H144, M1-R143, M1-E142, M1-V141, M1-  
A140, M1-I139, M1-C138, M1-T137, M1-M136, M1-T135, M1-L134, M1-I133, M1-  
20 E132, M1-T131, M1-V130, M1-V129, M1-A128, M1-T127, M1-S126, M1-Q125, M1-  
V124, M1-F123, M1-P122, M1-V121, M1-M120, M1-K119, M1-C118, M1-I117, M1-  
F116, M1-A115, M1-G114, M1-G113, M1-L112, M1-W111, M1-N110, M1-D109, M1-  
S108, M1-I107, M1-N106, M1-Q105, M1-L104, M1-M103, M1-T102, M1-V101, M1-  
25 P100, M1-I99, M1-C98, M1-F97, M1-F96, M1-T95, M1-I94, M1-L93, M1-L92, M1-  
D91, M1-S90, M1-L89, M1-A88, M1-L87, M1-S86, M1-C85, M1-I84, M1-F83, M1-I82,  
M1-N81, M1-T80, M1-V79, M1-T78, M1-R77, M1-M76, M1-A75, M1-K74, M1-S73,  
M1-R72, M1-T71, M1-V70, M1-V69, M1-Y68, M1-F67, M1-V66, M1-L65, M1-A64,  
M1-N63, M1-G62, M1-F61, M1-L60, M1-A59, M1-L58, M1-A57, M1-F56, M1-I55,  
30 M1-L54, M1-V53, M1-G52, M1-T51, M1-L50, M1-V49, M1-L48, M1-A47, M1-L46,  
M1-K45, M1-A44, M1-R43, M1-G42, M1-P41, M1-L40, M1-E39, M1-P38, M1-T37,  
M1-Y36, M1-V35, M1-L34, M1-P33, M1-R32, M1-L31, M1-R30, M1-Y29, M1-L28,  
M1-A27, M1-I26, M1-F25, M1-Q24, M1-E23, M1-R22, M1-T21, M1-L20, M1-N19,  
35 M1-H18, M1-D17, M1-R16, M1-L15, M1-L14, M1-R13, M1-S12, M1-F11, M1-Q10,  
M1-E9, M1-P8, and/or M1-T7 of SEQ ID NO:14. Polynucleotide sequences encoding  
these polypeptides are also provided. The present invention also encompasses the use of

these C-terminal HGPRBMY2 deletion polypeptides as immunogenic and/or antigenic  
5 epitopes as described elsewhere herein.

Figure 8 depicts the putative transmembrane regions of the HGPRBMY2 polypeptide as shaded areas of the sequence, and also presents a hydropathy plot which was used to predict the hydrophobic and hydrophilic regions of the full length polypeptide.  
10

The HGPRBMY2 sequence begins with a methionine in a DNA sequence context consistent with a translation initiation site. An alignment between the HGPRBMY2 polypeptide with neuropeptide, orexin and galanin receptor sequences is shown in Figure 9 (par2\_human, Genbank Accession No. gil18560788, SEQ ID NO:36; par3\_human, Genbank Accession No. NP\_004092, SEQ ID NO:37; thrombin\_Xeno, Genbank Accession No. gil2134162, SEQ ID NO:38; thrombin\_human, Genbank Accession No. NP\_001983, SEQ ID NO:39; par4\_human, Genbank Accession No. NP\_003941, SEQ ID NO:40; and p2y9\_human, Genbank Accession No. gil17426979, SEQ ID NO:41). Although the overall amino acid sequence identity between these molecules is low, there  
15 20 are numerous residues which are conserved across all of the GPCRs in the alignment suggesting a functional importance for that residue.

The HGPRBMY1 amino acid sequences of the invention include the amino acid sequence shown in Figure 2 (SEQ ID NO:2). The cDNA sequence (SEQ ID NO:1) described in Section 5.1 encodes the amino acid sequence of HGPRBMY1 (359 amino acids; SEQ ID NO:2). The extracellular domains ("ECD") of HGPRBMY1 extend from about amino acid residues 1 to about 27, about 85 to about 88, about 161 to about 186, and about 259 to about 276 of SEQ ID NO:2; the transmembrane domains ("TM") of HGPRBMY1 extend from about amino acid residues 28 to about 49, about 60 to about 84, about 89 to about 105, about 139 to about 160, about 187 to about 200, about 235 to about 258, and about 277 to about 297 of SEQ ID NO:2; and the cytoplasmic domains ("CD") of HGPRBMY1 extend from about amino acid residue 50 to about 59, about 106 to about 138, about 201 to about 234, and about 298 to about 359 of SEQ ID NO:2.  
25 30 35

Figure 3 depicts the putative transmembrane regions of the HGPRBMY1 polypeptide as shaded areas of the sequence, and also presents a hydropathy plot which was used to predict the hydrophobic and hydrophilic regions of the full length polypeptide.

The HGPRBMY1 sequence begins with a methionine in a DNA sequence context  
5 consistent with a translation initiation site. An alignment between the HGPRBMY1  
polypeptide with thrombin receptor, protease activated receptor (par) and P2Y9-like  
receptor sequences is shown in Figure 4 (OX2R\_HUMAN, Genbank Accession No.  
gil17978555, SEQ ID NO:42; OX2R\_RAT, Genbank Accession No. gil6981020, SEQ  
ID NO:43; NY4R\_MOUSE, Genbank Accession No. gil1587693, SEQ ID NO:44;  
10 NY4R\_RAT, Genbank Accession No. gil2494992, SEQ ID NO:45; NY6R\_RABIT,  
Genbank Accession No. gil3024242, SEQ ID NO:46; Q9WVD0, Genbank Accession No.  
gil5410446, SEQ ID NO:47; O57463, Genbank Accession No. gil2739141, SEQ ID  
NO:48; NY2R\_HUMAN, Genbank Accession No. NP\_000901, SEQ ID NO:49;  
15 Q9Y5X5, Genbank Accession No. gil4530469, SEQ ID NO:50; and GALR\_MOUSE,  
Genbank Accession No. gil3023827, SEQ ID NO:51. Although the overall amino acid  
sequence identity between these molecules is low, there are numerous residues which are  
conserved across all of the GPCRs in the alignment suggesting a functional importance  
for that residue.

20 Peptides and polypeptides of HGPRBMY1 or HGPRBMY2 or mutants thereof  
can also be chemically synthesized (*e.g.*, see Creighton, 1983, Proteins: Structures and  
Molecular Principles, W. H. Freeman & Co., N.Y.). In addition, polypeptides and  
peptides of the invention may be produced by recombinant DNA technology using  
25 techniques well known in the art for expressing nucleic acid containing HGPRBMY1 or  
HGPRBMY2 gene sequences and/or coding sequences. Such methods can be used to  
construct expression vectors containing various HGPRBMY1 or HGPRBMY2 nucleic  
acid sequences, including those described in Section 5.1, and appropriate transcriptional  
and translational control signals.

30 These constructs can be designed to encode and express polypeptides or peptides  
corresponding to one or more functional domains of the HGPRBMY1 or HGPRBMY2  
(*e.g.*, an ECD, a TM and/or a CD) in any order, truncated or deleted HGPRBMY1 or  
HGPRBMY2 (*e.g.*, HGPRBMY1 or HGPRBMY2 in which one or more TM and/or CD  
35 are deleted) as well as fusion polypeptides in which the HGPRBMY1 or HGPRBMY2  
or truncation/deletion mutant of HGPRBMY1 or HGPRBMY2 is fused to an unrelated  
polypeptide (*i.e.*, linked to a heterologous carrier polypeptide) and can be designed on the

basis of the HGPRBMY1 or HGPRBMY2 nucleic acid and HGPRBMY1 or  
5 HGPRBMY2 amino acid sequences disclosed in this Section and in Section 5.1, above.

The HGPRBMY1 or HGPRBMY2 polypeptide or peptide may be a soluble derivative, *e.g.*, HGPRBMY1 or HGPRBMY2 domains corresponding to one or more of the CD or ECD (*e.g.*, the four ECD constructed in frame and in tandem without linkers, or likewise the four CD in tandem, or any combination of soluble domains of the  
10 polypeptide of the invention); one or more of the ECD or CD linked via a hydrophilic peptide linker sequence and/or a flexible linker sequence (*e.g.*, such as GGSGG); or a truncated or deleted HGPRBMY1 or HGPRBMY2 in which the TM are deleted, the TM and CD are deleted or the TM and ECD are deleted, wherein the peptide or polypeptide  
15 can be recovered from the culture, *i.e.*, from the host cell in cases where the HGPRBMY1 or HGPRBMY2 peptide or polypeptide is not secreted, and from the culture media in cases where the HGPRBMY1 or HGPRBMY2 peptide or polypeptide is secreted by the cells. In a preferred embodiment, these polypeptides are soluble in normal physiological conditions.

20 Fusion polypeptides comprising HGPRBMY1 or HGPRBMY2 polypeptide or peptide sequences fused to heterologous sequences can include, but are not limited to, epitope tagged polypeptides or peptides, *e.g.*, GST fusions, Myc-tag, hemagglutinin-tag, histidine-tag, FLAG-tag, etc.; Ig-Fc fusions which stabilize the HGPRBMY1 or  
25 HGPRBMY2 polypeptide or peptide and prolong half-life *in vivo*; or fusions to any amino acid sequence that allows the fusion polypeptide to be anchored to the cell membrane, allowing the HGPRBMY1 or HGPRBMY2 domain to be exhibited on the cell surface. The fusion polypeptide can also be constructed with a protease cleavage site between the HGPRBMY1 or HGPRBMY2 and the heterologous sequences in order to  
30 allow release from the foreign sequences, *e.g.*, thrombin site or factor Xa.

The polypeptides or peptides of the invention can also be conjugated or fused to a compound, such as an enzyme, fluorescent polypeptide, or luminescent polypeptide which provide a marker function. Examples of suitable marker compounds include horseradish peroxidase, alkaline phosphatase,  $\beta$ -galactosidase, acetylcholinesterase,  
35 streptavidin/biotin, avidin/biotin, umbelliferone, fluorescein, fluorescein isothiocyanate, rhodamine, dichlorotriazinylamine fluorescein, dansyl chloride or phycoerythrin, luminol, luciferase, luciferin, aequorin,  $^{125}\text{I}$ ,  $^{131}\text{I}$ ,  $^{35}\text{S}$  or  $^3\text{H}$ .

Further, a polypeptide or peptide of the invention may be conjugated to a therapeutic moiety such as a cytotoxin, a therapeutic agent or a radioactive metal ion. A cytotoxin or cytotoxic agent includes any agent that is detrimental to cells. Examples include taxol, cytochalasin B, gramicidin D, ethidium bromide, emetine, mitomycin, etoposide, tenoposide, vincristine, vinblastine, colchicin, doxorubicin, daunorubicin, 5-dihydroxy anthracin dione, mitoxantrone, mithramycin, actinomycin D, 1-dehydrotestosterone, glucocorticoids, procaine, tetracaine, lidocaine, propranolol, and puromycin and analogs or homologues thereof. Therapeutic agents include, but are not limited to, antimetabolites (e.g., methotrexate, 6-mercaptopurine, 6-thioguanine, cytarabine, 5-fluorouracil decarbazine), alkylating agents (e.g., mechlorethamine, thioepa, chlorambucil, melphalan, carmustine (BSNU) and lomustine (CCNU), cyclothosphamide, busulfan, dibromomannitol, streptozotocin, mitomycin C, and cis-dichlorodiamine platinum (II) (DDP) cisplatin), anthracyclines (e.g., daunorubicin (formerly daunomycin) and doxorubicin), antibiotics (e.g., dactinomycin (formerly actinomycin), bleomycin, mithramycin, and anthramycin (AMC)), and anti-mitotic agents (e.g., vincristine and vinblastine).

In addition, a fusion polypeptide or peptide of the invention may be a conjugate or fusion with a drug moiety, which is not to be construed as limited to classical chemical therapeutic agents. For example, the drug moiety may be a polypeptide or polypeptide possessing a desired biological activity. Such polypeptides may include, for example, a toxin such as abrin, ricin A, pseudomonas exotoxin, or diphtheria toxin; a polypeptide such as tumor necrosis factor,  $\alpha$ -interferon,  $\beta$ -interferon, nerve growth factor, platelet derived growth factor, tissue plasminogen activator, a thrombotic agent or an anti-angiogenic agent, e.g., angiostatin or endostatin; or, biological response modifiers such as, for example, lymphokines, interleukin-1 ("IL-1"), interleukin-2 ("IL-2"), interleukin-4 ("IL-4"), interleukin-6 ("IL-6"), interleukin-7 ("IL-7"), granulocyte macrophage colony stimulating factor ("GM-CSF"), granulocyte colony stimulating factor ("G-CSF"), interleukin-10 ("IL-10"), interleukin-12 ("IL-12"), interleukin-17 ("IL-15"), interleukin-17 ("IL-17"), interferon- $\gamma$  ("IFN- $\gamma$ "), interferon- $\alpha$  ("IFN- $\alpha$ "), or other immune factors or growth factors.

Further, HGPRBMY1 or HGPRBMY2 polypeptides of other species are encompassed by the invention. In fact, any HGPRBMY1 or HGPRBMY2 polypeptide

encoded by the HGPRBMY1 or HGPRBMY2 nucleic acid sequences described in  
5 Section 5.1, above, are within the scope of the invention.

In another embodiment, polypeptides that are functionally equivalent to the HGPRBMY1 encoded by the nucleic acid sequences described in Section 5.1, as judged by any of a number of criteria, including but not limited to the ability to bind agonist or antagonist, the binding affinity for agonist or antagonist, the resulting biological effect  
10 of agonist or antagonist binding, *e.g.*, signal transduction, a change in cellular metabolism (*e.g.*, ion flux) or change in phenotype when the HGPRBMY1 equivalent is present in an appropriate cell type (such as the amelioration, prevention or delay of an immune disorder such as rheumatoid arthritis, leukemia or an immunodeficiency); by its ability  
15 to bind or compete with antibodies to HGPRBMY1 receptors; or by its ability to elicit antibodies that immunospecifically bind to the HGPRBMY1 receptor; etc.

The invention also encompasses polypeptides that are functionally equivalent to the HGPRBMY2 encoded by the nucleic acid sequences described in Section 5.1, as judged by any of a number of criteria, including but not limited to the ability to bind an  
20 antibody, an agonist or an antagonist, the binding affinity for agonist or antagonist, the resulting biological effect of agonist or antagonist binding, *e.g.*, signal transduction, a change in cellular metabolism (*e.g.*, ion flux, tyrosine phosphorylation) or change in phenotype when the HGPRBMY2 equivalent is present in an appropriate cell type (such as the amelioration, prevention or delay of congestive heart failure); by its ability to bind  
25 or compete with antibodies to HGPRBMY2 receptors; or by its ability to elicit antibodies that immunospecifically bind to the HGPRBMY2 receptor; etc.

Such functionally equivalent HGPRBMY1 polypeptides include but are not limited to additions or substitutions of amino acid residues within the amino acid  
30 sequence encoded by the HGPRBMY1 nucleic acid sequences described, above, in Section 5.1, but which result in a silent change, thus producing a functionally equivalent gene product. Amino acid substitutions may be made on the basis of similarity in polarity, charge, solubility, hydrophobicity, hydrophilicity, and/or the amphipathic nature of the residues involved. For example, nonpolar (hydrophobic) amino acids include alanine,  
35 leucine, isoleucine, valine, proline, phenylalanine, tryptophan, and methionine; polar neutral amino acids include glycine, serine, threonine, cysteine, tyrosine, asparagine, and glutamine; positively charged (basic) amino acids include arginine, lysine, and histidine;

and negatively charged (acidic) amino acids include aspartic acid and glutamic acid.  
5 Regional charge in the polypeptide can be determined analytically with computer programs, for example as shown in Figure 3, which depicts a hydropathy plot of the polypeptide sequence of Figure 2.

Such functionally equivalent HGPRBMY2 polypeptides include but are not limited to additions or substitutions of amino acid residues within the amino acid 10 sequence encoded by the HGPRBMY2 nucleic acid sequences described, above, in Section 5.1, but which result in a silent change, thus producing a functionally equivalent gene product. Amino acid substitutions may be made on the basis of similarity in polarity, charge, solubility, hydrophobicity, hydrophilicity, and/or the amphipathic nature of the 15 residues involved. For example, nonpolar (hydrophobic) amino acids include alanine, leucine, isoleucine, valine, proline, phenylalanine, tryptophan, and methionine; polar neutral amino acids include glycine, serine, threonine, cysteine, tyrosine, asparagine, and glutamine; positively charged (basic) amino acids include arginine, lysine, and histidine; and negatively charged (acidic) amino acids include aspartic acid and glutamic acid.  
20 Regional charges in the polypeptide can be determined analytically with computer programs, for example as shown in Figure 8, which depicts a hydropathy plot of the polypeptide sequence of Figure 7.

While random mutations can be made to HGPRBMY1 or HGPRBMY2 DNA and 25 the resulting mutant HGPRBMY1 or HGPRBMY2 tested for activity, site-directed mutations of the HGPRBMY1 or HGPRBMY2 coding sequence can be engineered using site-directed mutagenesis techniques known to those skilled in the art to generate a mutant HGPRBMY1 or HGPRBMY2 with modulated function, *e.g.*, higher binding affinity for agonist or antagonist, and/or changed signaling capacity, *e.g.*, lower binding 30 affinity for agonist or antagonist.

For example, the alignment of HGPRBMY1 and orexin is shown in Figure 4 in which identical amino acid residues are indicated by a black background. Mutant HGPRBMY1 can be engineered so that regions of identity (indicated by black 35 background in Figure 4) are maintained, whereas the variable residues (white background in Figure 4) are altered, *e.g.*, by deletion or insertion of an amino acid residue(s) or by substitution of one or more different amino acid residues. Conservative alterations at the variable positions can be engineered in order to produce a mutant HGPRBMY1 that

5 retains function; *e.g.*, agonist or antagonist binding affinity or signal transduction capability or both. Non-conservative changes can be engineered at these variable positions to alter function, *e.g.*, agonist or antagonist binding affinity or signal transduction capability, or both.

10 For example, the alignment of HGPRBMY2 and orexin is shown in Figure 9 in which identical amino acid residues are indicated by a black background. Mutant HGPRBMY2 can be engineered so that regions of identity (indicated by black background in Figure 9) are maintained, whereas the variable residues (white background in Figure 9) are altered, *e.g.*, by deletion or insertion of an amino acid residue(s) or by substitution of one or more different amino acid residues. Conservative alterations at the 15 variable positions can be engineered in order to produce a mutant HGPRBMY2 that retains function; *e.g.*, agonist or antagonist binding affinity or signal transduction capability or both. Non-conservative changes can be engineered at these variable positions to alter function, *e.g.*, agonist or antagonist binding affinity or signal transduction capability, or both.

20 In addition, mutation by deletion or non-conservative alteration of the conserved regions can be engineered where modulation of function is desired (*i.e.*, identical amino acids indicated by stars in Figure 4 or Figure 9). For example, deletion or non-conservative alterations (substitutions or insertions) of the agonist binding domain, 25 can be engineered to produce a mutant HGPRBMY1 or HGPRBMY2 that binds agonist or antagonist but is signaling-incompetent. Non-conservative alterations to the residues with a black background in the ECD shown in Figure 4 or Figure 9 can be engineered to produce mutant HGPRBMY1 or HGPRBMY2 with altered binding affinity for agonist or antagonist.

30 Other mutations to the HGPRBMY1 or HGPRBMY2 coding sequence can be made to generate HGPRBMY1 or HGPRBMY2 that are better suited for expression in host cells, *e.g.*, reduced toxicity, increased solubility, scale up, etc. in host cells. For example, cysteine residues can be deleted or substituted with another amino acid in order to eliminate disulfide bridges; N-linked glycosylation sites can be altered or eliminated 35 to achieve, for example, expression of a homogeneous product that is more easily recovered and purified from yeast hosts which are known to hyperglycosylate N-linked sites. To this end, a variety of amino acid substitutions at one or both of the first or third

amino acid positions of any one or more of the glycosylation recognition sequences 5 which occur in an ECD (N-X-S or N-X-T), and/or an amino acid deletion at the second position of any one or more such recognition sequences in the ECD will prevent glycosylation of the HGPRBMY1 or HGPRBMY2 at the modified tripeptide sequence. (See, *e.g.*, Miyajima et al., 1986, EMBO J. 5(6):1193-1197). In addition, the nucleic acid 10 construct can be designed to be polycistronic with alternative splice sites in order to increase production of polypeptides or peptides of the invention per cell, thus increasing yield.

The expression systems also encompass engineered host cells that express the HGPRBMY1 or HGPRBMY2 or functional equivalents *in situ*, *i.e.*, anchored in the cell 15 membrane. Purification or enrichment of the HGPRBMY1 or HGPRBMY2 from such expression systems can be accomplished using appropriate detergents and lipid micelles and methods well known to those skilled in the art. However, such engineered host cells themselves may be used in situations where it is important not only to retain the structural and functional characteristics of the HGPRBMY1 or HGPRBMY2, but to assess 20 biological activity, *e.g.*, in drug screening assays.

The expression systems that may be used for purposes of the invention include but are not limited to microorganisms such as bacteria (*e.g.*, *E. coli*, *B. subtilis*) transformed with recombinant bacteriophage DNA, plasmid DNA or cosmid DNA 25 expression vectors containing HGPRBMY1 or HGPRBMY2 nucleic acid sequences; yeast (*e.g.*, *Saccharomyces*, *Pichia*) transformed with recombinant yeast expression vectors containing the HGPRBMY1 or HGPRBMY2 nucleic acid sequences; insect cell systems infected with recombinant virus expression vectors (*e.g.*, baculovirus) containing the HGPRBMY1 or HGPRBMY2 sequences; plant cell systems infected with 30 recombinant virus expression vectors (*e.g.*, cauliflower mosaic virus, CaMV; tobacco mosaic virus, TMV) or transformed with recombinant plasmid expression vectors (*e.g.*, Ti plasmid) containing HGPRBMY1 or HGPRBMY2 nucleic acid sequences; or mammalian cell systems (*e.g.*, COS, CHO, BHK, 293, 3T3) harboring recombinant 35 expression constructs containing promoters derived from the genome of mammalian cells (*e.g.*, metallothionein promoter) or from mammalian viruses (*e.g.*, the adenovirus late promoter; the vaccinia virus 7.5K promoter).

In bacterial systems, a number of expression vectors may be advantageously selected depending upon the use intended for the HGPRBMY1 or HGPRBMY2 gene product being expressed. For example, when a large quantity of such a polypeptide is to be produced, for the generation of pharmaceutical compositions of HGPRBMY1 or HGPRBMY2 polypeptide or for raising antibodies to the HGPRBMY1 or HGPRBMY2 polypeptide, for example, vectors which direct the expression of high levels of fusion polypeptide products that are readily purified may be desirable. For example, pGEX vectors may also be used to express foreign polypeptides as fusion polypeptides with glutathione S-transferase (GST). The pGEX vectors are designed to include thrombin or factor Xa protease cleavage sites so that the cloned target gene product can be released from the GST moiety.

In an insect system, *Autographa californica* nuclear polyhedrosis virus (AcNPV) is used as a vector to express foreign genes. These recombinant viruses are then used to infect *Spodoptera frugiperda* cells in which the inserted gene is expressed. (e.g., see Smith et al., 1983, *J. Virol.* 46: 584; Smith, U.S. Pat. No. 4,215,051).

In mammalian host cells, a number of viral-based expression systems may be utilized. In adenovirus, the HGPRBMY1 or HGPRBMY2 nucleic acid sequence of interest may be ligated to an adenovirus transcription/translation control complex, e.g., the late promoter and tripartite leader sequence. This chimeric gene may then be inserted in the adenovirus genome by *in vitro* or *in vivo* recombination. Insertion in a non-essential region of the viral genome (e.g., region E1 or E3) will result in a recombinant virus that is viable and capable of expressing the HGPRBMY1 or HGPRBMY2 gene product in infected hosts (e.g., See Logan & Shenk, 1984, *Proc. Natl. Acad. Sci. USA* 81:3655-3659).

In cases where an entire HGPRBMY1 or HGPRBMY2 gene or cDNA, including its own initiation codon and adjacent sequences, is inserted into the appropriate expression vector, no additional translational control signals may be needed. However, in cases where only a portion of the HGPRBMY1 or HGPRBMY2 coding sequence is inserted, exogenous translational control signals, including, perhaps, the ATG initiation codon, must be provided. Furthermore, the initiation codon must be correctly oriented in the reading frame of the desired coding sequence to ensure translation of the insert in the correct reading frame. These exogenous translational control signals and initiation codons

can be of a variety of origins, both natural and synthetic. Intronic sequences and  
5 polyadenylation signals can also be included to increase the efficiency of expression.

In addition, a host cell strain may be chosen which modulates the expression of  
the inserted sequences, or modifies and processes the gene product in the specific fashion  
desired. Such modifications (e.g., glycosylation) and processing (e.g., cleavage) of  
10 polypeptide products may be important for the function of the polypeptide. Different host  
cells have characteristic and specific mechanisms for the post-translational processing  
and modification of polypeptides and gene products. Appropriate cell lines or host  
systems can be chosen to ensure the correct modification and processing of the foreign  
polypeptide expressed. To this end, eukaryotic host cells which possess the cellular  
15 machinery for proper processing of the primary transcript, glycosylation, and  
phosphorylation of the gene product may be used. Such mammalian host cells include but  
are not limited to CHO, VERO, BHK, HeLa, COS, MDCK, 293, 3T3, WI38, and in  
particular, bone marrow cell lines such as lymphocyte lineage (for example, monocyte,  
B-cell or T-cell, such as K562, WEHI 7.1 or WEHI-3 cell lines) or erythrocyte lineage  
20 cell lines.

For long-term, high-yield production of recombinant polypeptides, stable  
expression is preferred. For example, cell lines which stably express the HGPRBMY1  
or HGPRBMY2 sequences described above may be engineered. Rather than using  
25 expression vectors which contain viral origins of replication, host cells can be  
transformed with DNA controlled by appropriate expression control elements (e.g.,  
promoter, enhancer sequences, transcription terminators, polyadenylation sites, etc.), and  
a selectable marker. Following the introduction of the foreign DNA, engineered cells may  
be allowed to grow for 1-2 days in an enriched media, and then are switched to a selective  
30 media. The selectable marker in the recombinant plasmid confers resistance to the  
selection and allows cells to stably integrate the plasmid into their chromosomes and  
grow to form foci which in turn can be cloned and expanded into cell lines. This method  
may advantageously be used to engineer cell lines which express the HGPRBMY1 or  
35 HGPRBMY2 gene product. Such engineered cell lines may be particularly useful in  
screening and evaluation of compounds that affect the endogenous activity of the  
HGPRBMY1 or HGPRBMY2 gene product.

A number of selection systems may be used, including but not limited to the 5 herpes simplex virus thymidine kinase (tk) (Wigler, *et al.*, 1977, *Cell* 11:223), hypoxanthine-guanine phosphoribosyltransferase (hgprt) (Szybalska & Szybalski, 1962, *Proc. Natl. Acad. Sci. USA* 48:2026), and adenine phosphoribosyltransferase (aprt) (Lowy, *et al.*, 1980, *Cell* 22:817) genes can be employed in tk<sup>-</sup>, hgprt<sup>-</sup> or aprt<sup>-</sup> cells, 10 respectively. Also, antimetabolite resistance can be used as the basis of selection for the following genes: Dihydrofolate Reductase (DHFR), which confers resistance to methotrexate (Wigler, *et al.*, 1980, *Natl. Acad. Sci. USA* 77:3567; O'Hare, *et al.*, 1981, *Proc. Natl. Acad. Sci. USA* 78:1527); gpt, which confers resistance to mycophenolic acid (Mulligan & Berg, 1981, *Proc. Natl. Acad. Sci. USA* 78:2072); neomycin, which confers 15 resistance to the aminoglycoside G-418 (Colberre-Garapin, *et al.*, 1981, *J. Mol. Biol.* 150:1); and hygro, which confers resistance to hygromycin (Santerre, *et al.*, 1984, *Gene* 30:147).

The polypeptides of the invention can, for example, include modifications that 20 can increase such attributes as stability, half-life, ability to enter cells and aid in administration, *e.g.*, *in vivo* administration of the polypeptides of the invention. For example, polypeptides of the invention can comprise a polypeptide transduction domain of the HIV TAT polypeptide as described in Schwarze, *et al.* (1999 *Science* 285:1569-1572), thereby facilitating delivery of polypeptides of the invention into cells.

25 Alternatively, any fusion polypeptide may be readily purified by utilizing an antibody specific for the fusion polypeptide being expressed. For example, a system described by Janknecht *et al.* allows for the ready purification of non-denatured fusion polypeptides expressed in human cell lines (Janknecht, *et al.*, 1991, *Proc. Natl. Acad. Sci. USA* 88: 8972-8976). In this system, the gene of interest is subcloned into a vaccinia 30 recombination plasmid such that the gene's open reading frame is translationally fused to an amino-terminal tag consisting of six histidine residues. Extracts from cells infected with recombinant vaccinia virus are loaded onto Ni<sup>2+</sup> nitriloacetic acid-agarose columns and histidine-tagged polypeptides are selectively eluted with imidazole-containing buffers.

35 The HGPRBMY1 or HGPRBMY2 gene products can also be expressed in transgenic animals. Animals of any species, including, but not limited to, mice, rats, rabbits, guinea pigs, pigs, micro-pigs, goats, and non-human primates, *e.g.*, baboons,

monkeys, and chimpanzees may be used to generate HGPRBMY1 or HGPRBMY2  
5 transgenic animals.

Any technique known in the art may be used to introduce the HGPRBMY1 or HGPRBMY2 transgene into animals to produce the founder lines of transgenic animals. Such techniques include, but are not limited to pronuclear microinjection (Hoppe, P. C. and Wagner, T. E., 1989, U.S. Pat. No. 4,873,191); retrovirus mediated gene transfer into 10 germ lines (Van der Putten et al., 1985, Proc. Natl. Acad. Sci., USA 82:6148-6152); gene targeting in embryonic stem cells (Thompson et al., 1989, Cell 56:313-321); electroporation of embryos (Lo, 1983, Mol Cell. Biol. 3:1803-1814); and sperm-mediated gene transfer (Lavitrano et al., 1989, Cell 57:717-723); etc. For a review of such 15 techniques, see Gordon, 1989, Transgenic Animals, Intl. Rev. Cytol. 115:171-229, which is incorporated by reference herein in its entirety.

The present invention provides for transgenic animals that carry the HGPRBMY1 or HGPRBMY2 transgene in all their cells, as well as animals which carry the transgene in some, but not all their cells, *i.e.*, mosaic animals. The transgene may be integrated as 20 a single transgene or in concatamers, *e.g.*, head-to-head tandems or head-to-tail tandems. The transgene may also be selectively introduced into and activated in a particular cell type by following, for example, the teaching of Lasko et al. (Lasko, M. et al., 1992, Proc. Natl. Acad. Sci. USA 89: 6232-6236). The regulatory sequences required for such a 25 cell-type specific activation will depend upon the particular cell type of interest, and will be apparent to those of skill in the art. When it is desired that the HGPRBMY1 or HGPRBMY2 gene transgene be integrated into the chromosomal site of the endogenous HGPRBMY1 or HGPRBMY2 gene, gene targeting is preferred. Briefly, when such a technique is to be utilized, vectors containing some nucleic acid sequences homologous 30 to the endogenous HGPRBMY1 or HGPRBMY2 gene are designed for the purpose of integrating, via homologous recombination with chromosomal sequences, into and disrupting the function of the nucleic acid sequence of the endogenous HGPRBMY1 or HGPRBMY2 gene. The transgene may also be selectively introduced into a particular cell type, thus inactivating the endogenous HGPRBMY1 or HGPRBMY2 gene in only that 35 cell type, by following, for example, the teaching of Gu et al. (Gu, et al., 1994, Science 265: 103-106). The regulatory sequences required for such a cell-type specific

inactivation will depend upon the particular cell type of interest, and will be apparent to  
5 those of skill in the art.

Once transgenic animals have been generated, the expression of the recombinant HGPRBMY1 or HGPRBMY2 gene may be assayed utilizing standard techniques. Initial screening may be accomplished by Southern blot analysis or PCR techniques to analyze animal tissues to assay whether integration of the transgene has taken place. The level of  
10 mRNA expression of the transgene in the tissues of the transgenic animals may also be assessed using techniques which include but are not limited to Northern blot analysis of tissue samples obtained from the animal, *in situ* hybridization analysis, and RT-PCR. Samples of HGPRBMY1 or HGPRBMY2 gene-expressing tissue, may also be evaluated  
15 immunocytochemically using antibodies specific for the HGPRBMY1 or HGPRBMY2 transgene product.

### 5.3. Antibodies to HGPRBMY1

Antibodies that specifically recognize one or more epitopes of HGPRBMY1 or  
20 HGPRBMY2, or epitopes of conserved variants of HGPRBMY1 or HGPRBMY2 polypeptides or peptides, are also encompassed by the invention. Such antibodies include but are not limited to polyclonal antibodies, monoclonal antibodies (mAbs), humanized or chimeric antibodies, single chain antibodies, Fab fragments, F(ab')<sub>2</sub> fragments, fragments produced by a Fab expression library, anti-idiotypic (anti-Id) antibodies, and epitope-binding fragments of any of the above.

The antibodies of the invention may be used, for example, in the detection of the HGPRBMY1 in a biological sample and may, therefore, be utilized as part of a diagnostic or prognostic technique whereby patients may be tested for abnormal amounts of  
30 HGPRBMY1. Such antibodies may also be utilized in conjunction with, for example, compound screening schemes, as described, below, in Section 5.5, for the evaluation of the effect of test compounds on expression and/or activity of the HGPRBMY1 gene product. Additionally, such antibodies can be used in conjunction with the gene therapy techniques described, below, in Section 5.6, to, for example, evaluate the normal and/or  
35 engineered HGPRBMY1-expressing cells prior to their introduction into the patient. Such antibodies may additionally be used as a method for the inhibition of abnormal

HGPRBMY1 activity. Thus, such antibodies may, therefore, be utilized as part of 5 immune disorder treatment methods.

The antibodies of the invention may be used, for example, in the detection of the HGPRBMY2 in a biological sample and may, therefore, be utilized as part of a diagnostic or prognostic technique whereby patients may be tested for abnormal amounts of 10 HGPRBMY2. Such antibodies may also be utilized in conjunction with, for example, compound screening schemes, as described, below, in Section 5.5, for the evaluation of the effect of test compounds on expression and/or activity of the HGPRBMY2 gene product. Additionally, such antibodies can be used in conjunction with the gene therapy techniques described, below, in Section 5.6, to, for example, evaluate the normal and/or 15 engineered HGPRBMY2-expressing cells prior to their introduction into the patient. Such antibodies may additionally be used as a method for the inhibition of abnormal HGPRBMY2 activity. Thus, such antibodies may, therefore, be utilized as part of heart disorder treatment methods.

In a particular embodiment, HGPRBMY1 expression can be utilized as a marker 20 (*e.g.*, an *in situ* marker) for specific tissues (*e.g.*, bone marrow, spleen or thymus) and/or cells (*e.g.*, lymphocytes) in which HGPRBMY1 is expressed.

In a particular embodiment, HGPRBMY2 expression can be utilized as a marker 25 (*e.g.*, an *in situ* marker) for specific tissues (*e.g.*, heart, brain, etc.) and/or cells (*e.g.*, cells shown in Figures 10 and 16) in which HGPRBMY2 is expressed.

An isolated polypeptide or peptide of the invention can be used as an immunogen 30 to generate antibodies using standard techniques for polyclonal and monoclonal antibody preparation. The full-length polypeptide or a functional domain of the polypeptide, either native or denatured, can be used or, alternatively, the invention provides antigenic 35 polypeptides or peptides for use as immunogens. The antigenic peptide of a polypeptide of the invention comprises at least 8 (preferably 10, 15, 20, or 30) amino acid residues of the amino acid sequence of SEQ ID NO:2 or SEQ ID NO:14 or a variant thereof, and features an epitope of the polypeptide such that an antibody raised against the peptide forms a specific immune complex with the polypeptide, and alternatively with a native polypeptide.

Preferred epitopes encompassed by the antigenic peptide are regions that are located on the surface of the polypeptide, *e.g.*, hydrophilic regions, for example, as shown

in hydrophilic regions in Figure 3 or Figure 8. In certain embodiments, the nucleic acid 5 molecules of the invention are present as part of nucleic acid molecules comprising nucleic acid sequences that contain or encode heterologous (e.g., vector, expression vector, or fusion polypeptide) sequences. These nucleotides can then be used to express polypeptides which can be used as immunogens to generate an immune response, or more 10 particularly, to generate polyclonal or monoclonal antibodies specific to the expressed polypeptide.

An immunogen typically is used to prepare antibodies by immunizing a suitable subject, (e.g., rabbit, goat, mouse or other mammal). An appropriate immunogenic preparation can contain, for example, recombinantly expressed or chemically synthesized 15 polypeptide. The preparation can further include an adjuvant, such as Freund's complete or incomplete adjuvant, or similar immunostimulatory agent.

Accordingly, another aspect of the invention pertains to antibodies directed against a polypeptide of the invention. The term "antibody" as used herein refers to immunoglobulin molecules and immunologically active portions of immunoglobulin 20 molecules, *i.e.*, molecules that contain an antigen binding site which specifically binds an antigen, such as a polypeptide of the invention, *e.g.*, an epitope of a polypeptide of the invention. A molecule which specifically binds to a given polypeptide of the invention is a molecule which binds the polypeptide, but does not substantially bind other 25 molecules in a sample, *e.g.*, a biological sample, which naturally contains the polypeptide. Examples of immunologically active portions of immunoglobulin molecules include F(ab) and F(ab')<sub>2</sub> fragments which can be generated by treating the antibody with an enzyme such as pepsin. The invention provides polyclonal and monoclonal antibodies. The term "monoclonal antibody" or "monoclonal antibody composition", as used herein, 30 refers to a population of antibody molecules that contain only one species of an antigen binding site capable of immunoreacting with a particular epitope.

Polyclonal antibodies can be prepared by immunizing a suitable subject with a polypeptide of the invention as an immunogen. Preferred polyclonal antibody compositions are ones that have been selected for antibodies directed against a 35 polypeptide or polypeptides of the invention. Particularly preferred polyclonal antibody preparations are ones that contain only antibodies directed against a polypeptide or polypeptides of the invention. Particularly preferred immunogen compositions are those

that contain no other human polypeptides such as, for example, immunogen compositions  
5 made using a non-human host cell for recombinant expression of a polypeptide of the invention. In such a manner, the only human epitope or epitopes recognized by the resulting antibody compositions raised against this immunogen will be present as part of a polypeptide or polypeptides of the invention.

The antibody titer in the immunized subject can be monitored over time by  
10 standard techniques, such as with an enzyme linked immunosorbent assay (ELISA) using immobilized polypeptide. If desired, the antibody molecules can be isolated from the mammal (e.g., from the blood) and further purified by well-known techniques, such as protein A chromatography to obtain the IgG fraction. Alternatively, antibodies specific  
15 for a polypeptide or peptide of the invention can be selected for (e.g., partially purified) or purified by, e.g., affinity chromatography. For example, a recombinantly expressed and purified (or partially purified) polypeptide of the invention is produced as described herein, and covalently or non-covalently coupled to a solid support such as, for example, a chromatography column. The column can then be used to affinity purify antibodies  
20 specific for the polypeptides of the invention from a sample containing antibodies directed against a large number of different epitopes, thereby generating a substantially purified antibody composition, i.e., one that is substantially free of contaminating antibodies. By a substantially purified antibody composition is meant, in this context, that  
25 the antibody sample contains at most only 30% (by dry weight) of contaminating antibodies directed against epitopes other than those on the desired polypeptide or polypeptide of the invention, and preferably at most 20%, yet more preferably at most 10%, and most preferably at most 5% (by dry weight) of the sample is contaminating antibodies. A purified antibody composition means that at least 99% of the antibodies in  
30 the composition are directed against the desired polypeptide or peptide of the invention.

At an appropriate time after immunization, e.g., when the specific antibody titers are highest, antibody-producing cells can be obtained from the subject and used to prepare monoclonal antibodies by standard techniques, such as the hybridoma technique  
35 originally described by Kohler and Milstein (1975) *Nature* 256:495-497, the human B cell hybridoma technique (Kozbor et al. (1983) *Immunol. Today* 4:72), the EBV-hybridoma technique (Cole et al. (1985), *Monoclonal Antibodies and Cancer Therapy*, Alan R. Liss, Inc., pp. 77-96) or trioma techniques. The technology for

producing hybridomas is well known (see generally *Current Protocols in Immunology* 5 (1994) Coligan et al. (eds.) John Wiley & Sons, Inc., New York, NY). Hybridoma cells producing a monoclonal antibody of the invention are detected by screening the hybridoma culture supernatants for antibodies that bind the polypeptide of interest, e.g., using a standard ELISA assay.

Alternative to preparing monoclonal antibody-secreting hybridomas, a 10 monoclonal antibody directed against a polypeptide of the invention can be identified and isolated by screening a recombinant combinatorial immunoglobulin library (e.g., an antibody phage display library) with the polypeptide of interest. Kits for generating and screening phage display libraries are commercially available (e.g., the Pharmacia 15 *Recombinant Phage Antibody System*, Catalog No. 27-9400-01; and the Stratagene *SurfZAP Phage Display Kit*, Catalog No. 240612). Additionally, examples of methods and reagents particularly amenable for use in generating and screening antibody display library can be found in, for example, U.S. Patent No. 5,223,409; PCT Publication No. WO 92/18619; PCT Publication No. WO 91/17271; PCT Publication No. WO 92/20791; 20 PCT Publication No. WO 92/15679; PCT Publication No. WO 93/01288; PCT Publication No. WO 92/01047; PCT Publication No. WO 92/09690; PCT Publication No. WO 90/02809; Fuchs et al. (1991) *Bio/Technology* 9:1370-1372; Hay et al. (1992) *Hum. Antibod. Hybridomas* 3:81-85; Huse et al. (1989) *Science* 246:1275-1281; Griffiths 25 et al. (1993) *EMBO J.* 12:725-734.

Additionally, recombinant antibodies, such as chimeric and humanized monoclonal antibodies, comprising both human and non-human portions, which can be made using standard recombinant DNA techniques, are within the scope of the invention. A chimeric antibody is a molecule in which different portions are derived from different 30 animal species, such as those having a variable region derived from a murine mAb and a human immunoglobulin constant region. (See, e.g., Cabilly et al., U.S. Patent No. 4,816,567; and Boss et al., U.S. Patent No. 4,816,397, which are incorporated herein by reference in their entirety.) Humanized antibodies are antibody molecules from non-human species having one or more complementarily determining regions (CDRs) from 35 the non-human species and a framework region from a human immunoglobulin molecule (See, e.g., Queen, U.S. Patent No. 5,585,089, which is incorporated herein by reference in its entirety.) Such chimeric and humanized monoclonal antibodies can be produced by

recombinant DNA techniques known in the art, for example using methods described in  
5 PCT Publication No. WO 87/02671; European Patent Application 184,187; European  
Patent Application 171,496; European Patent Application 173,494; PCT Publication No.  
WO 86/01533; U.S. Patent No. 4,816,567; European Patent Application 125,023; Better  
et al. (1988) *Science* 240:1041-1043; Liu et al. (1987) *Proc. Natl. Acad. Sci. USA*  
84:3439-3443; Liu et al. (1987) *J. Immunol.* 139:3521-3526; Sun et al. (1987) *Proc. Natl.*  
10 *Acad. Sci. USA* 84:214-218; Nishimura et al. (1987) *Canc. Res.* 47:999-1005; Wood et  
al. (1985) *Nature* 314:446-449; and Shaw et al. (1988) *J. Natl. Cancer Inst.*  
80:1553-1559; Morrison (1985) *Science* 229:1202-1207; Oi et al. (1986) *Bio/Techniques*  
4:214; U.S. Patent 5,225,539; Jones et al. (1986) *Nature* 321:552-525; Verhoeven et al.  
15 (1988) *Science* 239:1534; and Beidler et al. (1988) *J. Immunol.* 141:4053-4060.

Completely human antibodies are particularly desirable for therapeutic treatment  
of human patients. Such antibodies can be produced, for example, using transgenic mice  
which are incapable of expressing endogenous immunoglobulin heavy and light chains  
genes, but which can express human heavy and light chain genes. The transgenic mice  
20 are immunized in the normal fashion with a selected antigen, e.g., all or a portion of a  
polypeptide of the invention. Monoclonal antibodies directed against the antigen can be  
obtained using conventional hybridoma technology. The human immunoglobulin  
transgenes harbored by the transgenic mice rearrange during B cell differentiation, and  
25 subsequently undergo class switching and somatic mutation. Thus, using such a  
technique, it is possible to produce therapeutically useful IgG, IgA and IgE antibodies.  
For an overview of this technology for producing human antibodies, see Lonberg and  
Huszar (1995, *Int. Rev. Immunol.* 13:65-93). For a detailed discussion of this technology  
for producing human antibodies and human monoclonal antibodies and protocols for  
30 producing such antibodies, see, e.g., U.S. Patent 5,625,126; U.S. Patent 5,633,425; U.S.  
Patent 5,569,825; U.S. Patent 5,661,016; and U.S. Patent 5,545,806. In addition,  
companies such as Abgenix, Inc. (Freemont, CA), can be engaged to provide human  
antibodies directed against a selected antigen using technology similar to that described  
above.

35 Completely human antibodies which recognize a selected epitope can be  
generated using a technique referred to as "guided selection." In this approach a selected  
non-human monoclonal antibody, e.g., a mouse antibody, is used to guide the selection

of a completely human antibody recognizing the same epitope (Jespers et al. (1994)  
5 *Bio/technology* 12:899-903).

An antibody directed against a polypeptide of the invention (*e.g.*, monoclonal antibody) can be used to isolate the polypeptide by standard techniques, such as affinity chromatography or immunoprecipitation. Moreover, such an antibody can be used to detect the polypeptide (*e.g.*, in a cellular lysate or cell supernatant) in order to evaluate  
10 the abundance and pattern of expression of the polypeptide. The antibodies can also be used diagnostically to monitor polypeptide levels in tissue as part of a clinical testing procedure, *e.g.*, to, for example, determine the efficacy of a given treatment regimen. Detection can be facilitated by coupling the antibody to a detectable substance. Examples  
15 of detectable substances include various enzymes, prosthetic groups, fluorescent materials, luminescent materials, bioluminescent materials, and radioactive materials. Examples of suitable enzymes include horseradish peroxidase, alkaline phosphatase,  $\beta$ -galactosidase, or acetylcholinesterase; examples of suitable prosthetic group complexes  
20 include streptavidin/biotin and avidin/biotin; examples of suitable fluorescent materials include umbelliferone, fluorescein, fluorescein isothiocyanate, rhodamine, dichlorotriazinylamine fluorescein, dansyl chloride or phycoerythrin; an example of a luminescent material includes luminol; examples of bioluminescent materials include  
25 luciferase, luciferin, and aequorin, and examples of suitable radioactive material include  $^{125}\text{I}$ ,  $^{131}\text{I}$ ,  $^{35}\text{S}$  or  $^3\text{H}$ .

In addition, the HGPRBMY1 or HGPRBMY2 gene sequences and gene products, including polypeptides, peptides, fusion polypeptides or peptides, and antibodies directed against said gene products and peptides, have applications for purposes independent of the role of the gene products. For example, HGPRBMY1 or HGPRBMY2 gene products,  
30 including polypeptides or peptides, as well as specific antibodies thereto, can be used for construction of fusion polypeptides to facilitate recovery, detection, or localization of another polypeptide of interest. In addition, HGPRBMY1 or HGPRBMY2 genes and gene products can be used for genetic mapping. Finally, HGPRBMY1 or HGPRBMY2  
35 nucleic acids and gene products have generic uses, such as supplemental sources of nucleic acids, polypeptides and amino acids for food additives or cosmetic products.

Further, an antibody of the invention (or fragment thereof) may be conjugated to a therapeutic moiety such as a cytotoxin, a therapeutic agent or a radioactive metal ion.

A cytotoxin or cytotoxic agent includes any agent that is detrimental to cells. Examples 5 include taxol, cytochalasin B, gramicidin D, ethidium bromide, emetine, mitomycin, etoposide, tenoposide, vincristine, vinblastine, colchicin, doxorubicin, daunorubicin, dihydroxy anthracin dione, mitoxantrone, mithramycin, actinomycin D, 1-dehydrotestosterone, glucocorticoids, procaine, tetracaine, lidocaine, propranolol, and 10 puromycin and analogs or homologues thereof. Therapeutic agents include, but are not limited to, antimetabolites (e.g., methotrexate, 6-mercaptopurine, 6-thioguanine, cytarabine, 5-fluorouracil decarbazine), alkylating agents (e.g., mechlorethamine, thioepa, chlorambucil, melphalan, carmustine (BSNU) and lomustine (CCNU), cyclophosphamide, busulfan, dibromomannitol, streptozotocin, mitomycin C, and cis-dichlorodiamine 15 platinum (II) (DDP) cisplatin), anthracyclines (e.g., daunorubicin (formerly daunomycin) and doxorubicin), antibiotics (e.g., dactinomycin (formerly actinomycin), bleomycin, mithramycin, and anthramycin (AMC)), and anti-mitotic agents (e.g., vincristine and vinblastine).

In addition, polypeptides, agonists or antagonists which bind a polypeptide of the 20 invention can also be conjugated to the foregoing, thereby targeting a toxin to cells expressing HGPRBMY1 or HGPRBMY2.

The conjugates of the invention can be used for modifying a given biological response, the drug moiety is not to be construed as limited to classical chemical 25 therapeutic agents. For example, the drug moiety may be a polypeptide or peptide possessing a desired biological activity. Such polypeptides may include, for example, a toxin such as abrin, ricin A, pseudomonas exotoxin, or diphtheria toxin; a polypeptide such as tumor necrosis factor,  $\alpha$ -interferon,  $\beta$ -interferon, nerve growth factor, platelet derived growth factor, tissue plasminogen activator, a thrombotic agent or an anti- 30 angiogenic agent, e.g., angiostatin or endostatin; or, biological response modifiers such as, for example, lymphokines, interleukin-1 ("IL-1"), interleukin-2 ("IL-2"), interleukin-4 ("IL-4"), interleukin-6 ("IL-6"), interleukin-7 ("IL-7"), granulocyte macrophage colony stimulating factor ("GM-CSF"), granulocyte colony stimulating factor ("G-CSF"), interleukin-10 ("IL-10"), interleukin-12 ("IL-12"), interleukin-17 ("IL-15"), 35 interleukin-17 ("IL-17"), interferon- $\gamma$  ("IFN- $\gamma$ "), interferon- $\alpha$  ("IFN- $\alpha$ "), or other immune factors or growth factors.

Techniques for conjugating such therapeutic moiety to antibodies are well known,  
5 see, *e.g.*, Arnon et al., "Monoclonal Antibodies For Immunotargeting Of Drugs In Cancer Therapy", in *Monoclonal Antibodies And Cancer Therapy*, Reisfeld et al. (eds.), pp. 243-56 (Alan R. Liss, Inc. 1985); Hellstrom et al., "Antibodies For Drug Delivery", in *Controlled Drug Delivery* (2nd Ed.), Robinson et al. (eds.), pp. 623-53 (Marcel Dekker, Inc. 1987); Thorpe, "Antibody Carriers Of Cytotoxic Agents In Cancer Therapy: A Review", in *Monoclonal Antibodies: Biological And Clinical Applications*, Pinchera et al. (eds.), pp. 475-506 (1985); "Analysis, Results, And Future Prospective Of The Therapeutic Use Of Radiolabeled Antibody In Cancer Therapy", in *Monoclonal Antibodies For Cancer Detection And Therapy*, Baldwin et al. (eds.), pp. 303-16 (Academic Press 1985), and Thorpe et al., "The Preparation And Cytotoxic Properties Of Antibody-Toxin Conjugates", *Immunol. Rev.*, 62:119-58 (1982).

An antibody with or without a therapeutic moiety conjugated to it can be used as a therapeutic that is administered alone or in combination with chemotherapeutic agents.

20 Alternatively, an antibody of the invention can be conjugated to a second antibody to form an "antibody heteroconjugate" as described by Segal in U.S. Patent No. 4,676,980 or alternatively, the antibodies can be conjugated to form an "antibody heteropolymer" as described in Taylor *et al.*, in U.S. Patent Nos. 5,470,570 and 5,487,890.

25 An antibody with or without a therapeutic moiety conjugated to it can be used as a therapeutic that is administered alone or in combination with cytotoxic factor(s) and/or cytokine(s).

30 In yet a further aspect, the invention provides substantially purified antibodies or fragments thereof, including human or non-human antibodies or fragments thereof, which antibodies or fragments specifically bind to a polypeptide of the invention comprising an amino acid sequence of SEQ ID NO:2 or SEQ ID NO:14 or a variant thereof. In various embodiments, the substantially purified antibodies of the invention, or fragments thereof, can be human, non-human, chimeric and/or humanized antibodies.

35 In another aspect, the invention provides human or non-human antibodies or fragments thereof, which antibodies or fragments specifically bind to a polypeptide comprising an amino acid sequence of SEQ ID NO:2 or SEQ ID NO:14 or a variant thereof. Such non-human antibodies can be goat, mouse, sheep, horse, chicken, rabbit,

or rat antibodies. Alternatively, the non-human antibodies of the invention can be  
5 chimeric and/or humanized antibodies. In addition, the non-human antibodies of the  
invention can be polyclonal antibodies or monoclonal antibodies.

In still a further aspect, the invention provides monoclonal antibodies or  
fragments thereof, which antibodies or fragments specifically bind to a polypeptide of the  
10 invention comprising an amino acid sequence of SEQ ID NO:2 or SEQ ID NO:14 or a  
variant thereof. The monoclonal antibodies can be human, humanized, chimeric and/or  
non-human antibodies.

The substantially purified antibodies or fragments thereof specifically bind to a  
signal peptide, a secreted sequence, an extracellular domain, a transmembrane or a  
15 cytoplasmic domain cytoplasmic membrane of a polypeptide of the invention. In a  
particularly preferred embodiment, the substantially purified antibodies or fragments  
thereof, the non-human antibodies or fragments thereof, and/or the monoclonal antibodies  
or fragments thereof, of the invention specifically bind to a secreted sequence, or  
alternatively, to an extracellular domain of the amino acid sequence of the invention..

20 Any of the antibodies of the invention can be conjugated to a therapeutic moiety  
or to a detectable substance. Non-limiting examples of detectable substances that can be  
conjugated to the antibodies of the invention are an enzyme, a prosthetic group, a  
fluorescent material, a luminescent material, a bioluminescent material, and a radioactive  
25 material.

The invention also provides a kit containing an antibody of the invention  
conjugated to a detectable substance, and instructions for use. Still another aspect of the  
invention is a pharmaceutical composition comprising an antibody of the invention and  
a pharmaceutically acceptable carrier. In preferred embodiments, the pharmaceutical  
30 composition contains an antibody of the invention, a therapeutic moiety, and a  
pharmaceutically acceptable carrier.

Still another aspect of the invention is a method of making an antibody that  
specifically recognizes HGPRBMY1 or HGPRBMY2, the method comprising  
35 immunizing a mammal with a polypeptide. After immunization, a sample is collected  
from the mammal that contains an antibody that specifically recognizes the immunogen.  
Preferably, the polypeptide is recombinantly produced using a non-human host cell.  
Optionally, the antibodies can be further purified from the sample using techniques well

known to those of skill in the art. The method can further comprise producing a 5 monoclonal antibody-producing cell from the cells of the mammal. Optionally, antibodies are collected from the antibody-producing cell.

Alternatively, techniques described for the production of single chain antibodies (U.S. Pat. No. 4,946,778; Bird, 1988, *Science* 242:423-426; Huston et al., 1988, *Proc. 10 Natl. Acad. Sci. USA* 85:5879-5883; and Ward et al., 1989, *Nature* 334:544-546) can be adapted to produce single chain antibodies against HGPRBMY1 or HGPRBMY2 gene products. Single chain antibodies are formed by linking the heavy and light chain fragments of the Fv region via an amino acid bridge, resulting in a single chain 15 polypeptide.

Antibodies to the HGPRBMY1 can, in turn, be utilized to generate anti-idiotype antibodies that "mimic" the HGPRBMY1, using techniques well known to those skilled in the art (See, e.g., Greenspan & Bona, 1993, *FASEB J* 7(5):437-444; and Nissinoff, 1991, *J. Immunol.* 147(8):2429-2438). For example antibodies which bind to the 20 HGPRBMY1 ECD and competitively inhibit the binding of agonist or antagonist to the HGPRBMY1 can be used to generate anti-idiotypes that "mimic" the ECD and, therefore, bind and neutralize agonist or antagonist. Such neutralizing anti-idiotypes or Fab fragments of such anti-idiotypes can be used in therapeutic regimens to neutralize agonist or antagonist and prevent immune disorders.

Antibodies to the HGPRBMY2 can, in turn, be utilized to generate anti-idiotype antibodies that "mimic" the HGPRBMY2, using techniques well known to those skilled in the art. (See, e.g., Greenspan & Bona, 1993, *FASEB J* 7(5):437-444; and Nissinoff, 1991, *J. Immunol.* 147(8):2429-2438). For example antibodies which bind to the 25 HGPRBMY2 ECD and competitively inhibit the binding of agonist or antagonist to the HGPRBMY2 can be used to generate anti-idiotypes that "mimic" the ECD and, therefore, bind and neutralize agonist or antagonist. Such neutralizing anti-idiotypes or Fab fragments of such anti-idiotypes can be used in therapeutic regimens to neutralize agonist or antagonist and prevent heart failure or neural disorders.

35 **5.4. Diagnosis of Immune Disorders**

5 A variety of methods can be employed for the diagnostic and prognostic evaluation of immune disorders and for the identification of subjects having a predisposition to such disorders.

10 Immune system disorders occur when the immune response is inappropriate, excessive, or lacking. Immunodeficiency disorders occur when the immune system fails to fight tumors or invading substances. This causes persistent or recurrent infections, severe infections by organisms that are normally mild, incomplete recovery from illness or poor response to treatment, and increased incidence of cancer and other tumors. Opportunistic infections are widespread infections by microorganisms that are usually controllable.

15 People are said to be "immunosuppressed" when they experience immunodeficiency that is caused by medications such as corticosteroids or immunosuppressant (chemotherapy) medications. This is a desired part of treatment for disorders such as autoimmune disorders. It is used after organ transplantation to prevent transplant rejection. Acquired immunodeficiency may be a complication of diseases such 20 as HIV, infection and AIDS (acquired immunodeficiency syndrome), or from malnutrition.

25 Various immune disorders include, but are not limited to: congenital immunodeficiency, Anemia, Antiphospholipid Syndrome (APS), Blue Rubber Bleb Nevus Syndrome, Gout, Hemophilia, Leukemia, Myeloproliferative Disorders, Sickle Cell Disease, and Thalassemia. Additionally, diseases which affect immune function are contemplated, for example those that cause immunodeficiency such as AIDS/HIV.

30 Examples of congenital immunodeficiency disorders of antibody production (B lymphocyte abnormalities) include hypo-gammaglobulinemia (lack of one or more specific antibodies), which usually causes repeated mild respiratory infections, and agammaglobulinemia (lack of all or most antibody production), which results in frequent severe infections and is often fatal. Congenital disorders affecting the T lymphocytes may cause increased susceptibility to fungi, resulting in repeated Candida (yeast) infections.

35 Inherited combined immunodeficiency affects both T lymphocytes and B lymphocytes.

35 The following conditions and diseases often result in an immunodeficient state: ataxia-telangiectasia, DiGeorge syndrome, Chediak-Higashi syndrome, Job syndrome, leukocyte adhesion defects, panhypogammaglobulinemia, Bruton disease, congenital

agammaglobulinemia, selective deficiency of IgA, combined immunodeficiency disease,  
5 Wiscott-Aldrich syndrome, and complement deficiencies.

Suppression of the immune system may be desired in the treatment of certain disorders, or it may be a side effect of some treatments, for example in organ or bone marrow transplantation.

10 Immune deficiency is identified partly by poor response to treatment, delayed or incomplete recovery from illness, the presence of certain types of cancers (such as Kaposi's sarcoma), opportunistic infections (such as widespread *Pneumocystis carinii* infection or recurrent fungal/yeast infections).

15 Autoimmune disorders occur when the normal control process is disrupted. They may also occur if normal body tissue is altered so that it is no longer recognized as "self." Because autoimmune disorders and allergy are both caused by hypersensitivity reactions, it is believed that a history of allergy indicates increased risk for autoimmune disorders.

20 Examples of autoimmune (or autoimmune-related) disorders include but are not limited to: Hashimoto's thyroiditis, pernicious anemia, Addison's disease, diabetes mellitus, rheumatoid arthritis, systemic lupus erythematosus, dermatomyositis, Sjogren's syndrome, dermatomyositis, lupus erythematosus, multiple sclerosis, myasthenia gravis, Reiter's syndrome, and Graves disease.

25 Additional immune disorders include: Giant Lymph Node Hyperplasia, Castleman disease, Small Bowel Nodules, Immunoblastic Lymphadenopathy, Immunoproliferative Small Intestinal Disease, myelodysplasia syndrome 1, Still's syndrome, Lymphangiomyoma, Lymphoma, Abdominal Visceral Lymphoma, Bilaterally Large Multifocal Kidneys, Marek's Disease, Sezary Syndrome, Mycosis Fungoides and Tumor Lysis Syndrome.

30 Organs and tissues commonly affected by autoimmune disorders include blood components such as red blood cells, blood vessels, connective tissues, endocrine glands such as the thyroid or pancreas, muscles, joints, and skin. A person may experience more than one autoimmune disorder at the same time. Some disorders have multiple interrelated causes, one of which is autoimmunity.

35 Leukemias are defined generally as a group of usually fatal diseases of the reticuloendothelial system involving uncontrolled proliferation of white blood cells (leukocytes) such as: chronic myelogenous leukemia (CML), hairy cell leukemia, chronic

lymphocytic leukemia (CLL), acute lymphocytic leukemia, acute nonlymphocytic  
5 leukemia (AML), and chronic myelomonocytic leukemia.

Moreover, as the compositions of the invention relate to bone marrow, it is also contemplated that BMPRBMY1 can be targeted for modulation of anemia. Anemias which can be treated by methods of the invention include but are not limited to: anemia of B12 deficiency, anemia of chronic disease, anemia of folate deficiency, drug-induced  
10 immune hemolytic anemia, hemolytic anemia, hemolytic anemia due to g6pd deficiency, idiopathic aplastic anemia, idiopathic autoimmune hemolytic anemia, immune hemolytic anemia, iron deficiency anemia, megaloblastic anemia, pernicious anemia, secondary aplastic anemia, and sickle cell anemia.

15 Other HGPRBMY1 associated disorders can include TNF related disorders (e.g., acute myocarditis, myocardial infarction, congestive heart failure, T cell disorders (e.g., dermatitis, fibrosis)), immunological differentiative and apoptotic disorders (e.g., hyper-proliferative syndromes such as systemic lupus erythematosus (lupus)), and disorders related to angiogenesis (e.g., tumor formation and/or metastasis, cancer). Modulators of  
20 HGPRBMY1 expression and/or activity can be used to treat such disorders.

Methods of diagnosing or detecting immune disorders may, for example, utilize reagents such as the HGPRBMY1 nucleic acid sequences described in Section 5.1, and HGPRBMY1 antibodies, as described, in Section 5.3. Specifically, such reagents may be used, for example, for: (1) the detection of the presence of HGPRBMY1 gene mutations,  
25 or the detection of either over- or under-expression of HGPRBMY1 mRNA relative to the non-immune related disorder state; (2) the detection of either an over- or an under-abundance of HGPRBMY1 gene product relative to the non-immune related disorder state; and (3) the detection of perturbations or abnormalities in the signal  
30 transduction pathway mediated by HGPRBMY1.

The methods described herein may be performed, for example, by utilizing pre-packaged diagnostic kits comprising at least one specific HGPRBMY1 nucleic acid sequence or HGPRBMY1 antibody reagent described herein, which may be conveniently used, e.g., in clinical settings, to diagnose patients exhibiting immune related disorder  
35 abnormalities.

For the detection of HGPRBMY1 mutations, any nucleated cell can be used as a starting source for genomic nucleic acid. For the detection of HGPRBMY1 gene

expression or HGPRBMY1 gene products, any cell type or tissue in which the  
5 HGPRBMY1 gene is expressed, such as, for example, immune cells, may be utilized.

Nucleic acid-based detection techniques are described, below, in Section 5.4.1.

Peptide detection techniques are described, below, in Section 5.4.2.

#### 5.4b. Diagnosis of Cardiovascular Disorders

10

A variety of methods can be employed for the diagnostic and prognostic evaluation of HGPRBMY2-related cardiovascular disorders and for the identification of subjects having a predisposition to such disorders. Various forms of heart disease include: cardiomyopathy, aortic valve prolapse; aortic valve stenosis; arrhythmia; 15 cardiogenic shock; congenital heart disease; heart attack; heart failure; heart tumor; heart valve pulmonary stenosis; idiopathic cardiomyopathy; ischemic cardiomyopathy; mitral regurgitation (acute); mitral regurgitation (chronic); mitral stenosis; mitral valve prolapse; stable angina; hypotension; hypertension; acute heart failure; angina pectoris; and tricuspid regurgitation.

20

Congestive heart failure may affect either the right side, left side, or both sides of the heart. As pumping action is lost, blood may back up into other areas of the body, including the liver, gastrointestinal tract, and extremities (right-sided heart failure), or the lungs (left-sided heart failure).

25

Structural or functional causes of heart failure include high blood pressure (hypertension), heart valve disease, congenital heart diseases, cardiomyopathy, heart tumor, and other heart diseases. Precipitating factors include infections with high fever or complicated infections, use of negative inotropic drugs (such as  $\beta$ -blocker and calcium channel blocker), anemia, irregular heartbeats (arrhythmia), hyperthyroidism, and kidney 30 disease.

35

Furthermore, cardiomyopathy is a disease affecting the heart muscle (myocardium); this disease usually results in the inadequate heart pumping. Causes, incidence, and risk factors for cardiomyopathy include: viral infections; heart attacks; alcoholism; long-term, severe high blood pressure (hypertension); or for other reasons not yet known. Specific types of cardiomyopathy include: ischemic cardiomyopathy; idiopathic cardiomyopathy; hypertrophic cardiomyopathy; alcoholic cardiomyopathy; peripartum cardiomyopathy; dilated cardiomyopathy; and restrictive cardiomyopathy.

Cardiomyopathy is not common but can be severely disabling or fatal. Extreme 5 cardiomyopathy with heart failure may require a heart transplant.

Methods of diagnosing or detecting heart diseases may, for example, utilize reagents such as the HGPRBMY2 nucleic acid sequences described in Section 5.1, and HGPRBMY2 antibodies, as described, in Section 5.3. Specifically, such reagents may be used, for example, for: (1) the detection of the presence of HGPRBMY2 gene mutations, 10 or the detection of either over- or under-expression of HGPRBMY2 mRNA relative to the non-cardiovascular disorder state; (2) the detection of either an over- or an under-abundance of HGPRBMY2 gene product relative to the non-cardiovascular disorder state; and (3) the detection of perturbations or abnormalities in the signal 15 transduction pathway mediated by HGPRBMY2.

The methods described herein may be performed, for example, by utilizing pre-packaged diagnostic kits comprising at least one specific HGPRBMY2 nucleic acid sequence or HGPRBMY2 antibody reagent described herein, which may be conveniently used, *e.g.*, in clinical settings, to diagnose patients exhibiting cardiovascular disorder 20 abnormalities.

For the detection of HGPRBMY2 mutations, any nucleated cell can be used as a starting source for genomic nucleic acid. For the detection of HGPRBMY2 gene expression or HGPRBMY2 gene products, any cell type or tissue in which the 25 HGPRBMY2 gene is expressed, such as, for example, heart cells, may be utilized.

Nucleic acid-based detection techniques are described, below, in Section 5.4.1. Peptide detection techniques are described, below, in Section 5.4.2.

#### **5.4.1. Detection of the HGPRBMY1 and HGPRBMY2 Gene and Transcripts**

30 Mutations within the HGPRBMY1 or HGPRBMY2 gene can be detected by utilizing a number of techniques. Nucleic acid from any nucleated cell can be used as the starting point for such assay techniques, and may be isolated according to standard nucleic acid preparation procedures which are well known to those of skill in the art.

35 DNA may be used in hybridization or amplification assays of biological samples to detect abnormalities involving HGPRBMY1 or HGPRBMY2 gene structure, including point mutations, insertions, deletions and chromosomal rearrangements. Such assays may

5 include, but are not limited to, Southern analyses, single stranded conformational polymorphism analyses (SSCP), and PCR analyses.

Such diagnostic methods for the detection of HGPRBMY1 or HGPRBMY2 gene-specific mutations can involve for example, contacting and incubating nucleic acids including recombinant DNA molecules, cloned genes or degenerate variants thereof, obtained from a sample, *e.g.*, derived from a patient sample or other appropriate cellular 10 source, with one or more labeled nucleic acid reagents including recombinant DNA molecules, cloned genes or degenerate variants thereof, as described in Section 5.1, under conditions favorable for the specific annealing of these reagents to their complementary sequences within the HGPRBMY1 or HGPRBMY2 gene. Preferably, the lengths of these 15 nucleic acid reagents are at least 15 to 30 nucleotides. After incubation, all non-annealed nucleic acids are removed from the nucleic acid:HGPRBMY1 or HGPRBMY2 molecule hybrid.

20 The presence of nucleic acids which have hybridized, if any such molecules exist, is then detected. Using such a detection scheme, the nucleic acid from the cell type or tissue of interest can be immobilized, for example, to a solid support such as a membrane, or a plastic surface such as that on a microtiter plate or polystyrene beads. In this case, after incubation, non-annealed, labeled nucleic acid reagents of the type 25 described in Section 5.1 are easily removed. Detection of the remaining, annealed, labeled HGPRBMY1 or HGPRBMY2 nucleic acid reagents is accomplished using standard techniques well-known to those in the art. The HGPRBMY1 or HGPRBMY2 gene sequences to which the nucleic acid reagents have annealed can be compared to the annealing pattern expected from a normal HGPRBMY1 or HGPRBMY2 gene sequence in order to determine whether an HGPRBMY1 or HGPRBMY2 gene mutation is present.

30 Alternative diagnostic methods for the detection of HGPRBMY1 or HGPRBMY2 gene specific nucleic acid molecules, in patient samples or other appropriate cell sources, may involve their amplification, *e.g.*, by PCR (the experimental embodiment set forth in Mullis, K. B., 1987, U.S. Pat. No. 4,683,202), followed by the detection of the amplified 35 molecules using techniques well known to those of skill in the art. The resulting amplified sequences can be compared to those which would be expected if the nucleic acid being amplified contained only normal copies of the HGPRBMY1 or HGPRBMY2

5 gene in order to determine whether an HGPRBMY1 or HGPRBMY2 gene mutation exists.

10 Additionally, well-known genotyping techniques can be performed to identify individuals carrying HGPRBMY1 or HGPRBMY2 gene mutations. Such techniques include, for example, the use of restriction fragment length polymorphisms (RFLPs), which involve sequence variations in one of the recognition sites for the specific restriction enzyme used.

15 Additionally, improved methods for analyzing DNA polymorphisms which can be utilized for the identification of HGPRBMY1 or HGPRBMY2 gene mutations have been described which capitalize on the presence of variable numbers of short, tandemly repeated DNA sequences between the restriction enzyme sites. For example, Weber (U.S. Pat. No. 5,075,217, which is incorporated herein by reference in its entirety) describes a DNA marker based on length polymorphisms in blocks of (dC-dA)n-(dG-dT)n short tandem repeats. The average separation of (dC-dA)n-(dG-dT)n blocks, is estimated to be 20 30,000-60,000 bp. Markers which are so closely spaced exhibit a high frequency co-inheritance, and are extremely useful in the identification of genetic mutations, such as, for example, mutations within the HGPRBMY1 or HGPRBMY2 gene, and the diagnosis of diseases and disorders related to HGPRBMY1 or HGPRBMY2 mutations.

25 A DNA profiling assay for detecting short tri and tetra nucleotide repeat sequences has been described (U.S. Pat. No. 5,364,759, which is incorporated herein by reference in its entirety). This process includes extracting the DNA of interest, such as the HGPRBMY1 or HGPRBMY2 gene, amplifying the extracted DNA, and labeling the repeat sequences to form a genotypic map of the individual's DNA.

30 The level of HGPRBMY1 or HGPRBMY2 gene expression can also be assayed by detecting and measuring HGPRBMY1 or HGPRBMY2 transcription. For example, RNA from a cell type or tissue known, or suspected to express the HGPRBMY1 or HGPRBMY2 gene, such as bone marrow or spleen cells, may be isolated and tested utilizing hybridization or PCR techniques such as are described, above. The isolated cells can be derived from cell culture or from a patient. The analysis of cells taken from culture 35 may be a necessary step in the assessment of cells to be used as part of a cell-based gene therapy technique or, alternatively, to test the effect of compounds on the expression of the HGPRBMY1 or HGPRBMY2 gene. Such analyses may reveal both quantitative and

qualitative aspects of the expression pattern of the HGPRBMY1 or HGPRBMY2 gene,  
5 including activation or inactivation of HGPRBMY1 or HGPRBMY2 gene expression.

In one embodiment of such a detection scheme, cDNAs are synthesized from the RNAs of interest (e.g., by reverse transcription of the RNA molecule into cDNA). A sequence within the cDNA is then used as the template for a nucleic acid amplification reaction, such as a PCR amplification reaction, or the like. The nucleic acid reagents used  
10 as synthesis initiation reagents (e.g., primers) in the reverse transcription and nucleic acid amplification steps of this method are chosen from among the HGPRBMY1 or HGPRBMY2 nucleic acid reagents described in Section 5.1. The preferred lengths of such nucleic acid reagents are at least 9-30 nucleotides. For detection of the amplified  
15 product, the nucleic acid amplification may be performed using radioactively or non-radioactively labeled nucleic acids. Alternatively, enough amplified product may be made such that the product may be visualized by standard ethidium bromide staining or by utilizing any other suitable nucleic acid staining method.

Additionally, it is possible to perform such HGPRBMY1 or HGPRBMY2 gene  
20 expression assays "in situ", i.e., directly upon tissue sections (fixed and/or frozen) of patient tissue obtained from biopsies or resections, such that no nucleic acid purification is necessary. Nucleic acid reagents such as those described in Section 5.1 may be used as probes and/or primers for such *in situ* procedures (See, for example, Nuovo, G. J.,  
25 1992, "PCR In Situ Hybridization: Protocols And Applications", Raven Press, NY).

Alternatively, if a sufficient quantity of the appropriate cells can be obtained, standard Northern analysis can be performed to determine the level of mRNA expression of the HGPRBMY1 or HGPRBMY2 gene.

### 30 5.4.2. Detection of the HGPRBMY1 and HGPRBMY2 Gene Products

Antibodies directed against wild type or mutant HGPRBMY1 or HGPRBMY2 gene products or conserved variants of the polypeptides or peptides, which are discussed, above, in Section 5.3, may also be used as immune related disorder diagnostics and prognostics, as described herein. Such diagnostic methods, may be used to detect  
35 abnormalities in the level of HGPRBMY1 or HGPRBMY2 gene expression, or abnormalities in the structure and/or temporal, tissue, cellular, or subcellular location of

the HGPRBMY1 or HGPRBMY2, and may be performed *in vivo* or *in vitro*, such as, for 5 example, on biopsy tissue.

For example, antibodies directed to epitopes of the HGPRBMY1 or HGPRBMY2 ECD can be used *in vivo* to detect the pattern and level of expression of the HGPRBMY1 or HGPRBMY2 in the body. Such antibodies can be labeled, *e.g.*, with a radio-opaque or other appropriate compound and injected into a subject in order to visualize binding 10 to the HGPRBMY1 or HGPRBMY2 expressed in the body using methods such as X-rays, CAT-scans, or MRI. Labeled antibody fragments, *e.g.*, the Fab or single chain antibody comprising the smallest portion of the antigen binding region, are preferred for maximum labeling of HGPRBMY1 or HGPRBMY2 expressed in the bone marrow or 15 spleen.

Additionally, any HGPRBMY1 or HGPRBMY2 fusion polypeptide or HGPRBMY1 or HGPRBMY2 conjugated polypeptide whose presence can be detected, can be administered. For example, HGPRBMY1 or HGPRBMY2 fusion or conjugated polypeptides labeled with a radio-opaque or other appropriate compound can be 20 administered and visualized *in vivo* for labeled antibodies. Further such agonist or antagonist fusion polypeptides as AP-GPCR on GPCR-Ap fusion polypeptides can be utilized for *in vitro* diagnostic procedures. Alternatively, immunoassays or fusion polypeptide detection assays, can be utilized on biopsy and autopsy samples *in vitro* to 25 permit assessment of the expression pattern of the HGPRBMY1 or HGPRBMY2. Such assays are not confined to the use of antibodies that define the HGPRBMY1 or HGPRBMY2 ECD, but can include the use of antibodies directed to epitopes of any of the domains of the HGPRBMY1 or HGPRBMY2, *e.g.*, the ECD, the TM and/or CD. The use of each or all of these labeled antibodies will yield useful information regarding 30 translation and intracellular transport of the HGPRBMY1 or HGPRBMY2 to the cell surface, and can identify defects in processing.

The tissue or cell type to be analyzed will generally include those which are known, or suspected, to express the HGPRBMY1 or HGPRBMY2 gene, such as, for 35 example, bone marrow or spleen cells. The polypeptide isolation methods employed herein may, for example, be such as those described in Harlow and Lane (Harlow, E. and Lane, D., 1988, "Antibodies: A Laboratory Manual", Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.), which is incorporated herein by reference in its

entirety. The isolated cells can be derived from cell culture or from a patient. The analysis  
5 of cells taken from culture may be a necessary step in the assessment of cells that could  
be used as part of a cell-based gene therapy technique or, alternatively, to test the effect  
of compounds on the expression of the HGPRBMY1 or HGPRBMY2 gene. For example,  
10 antibodies, or fragments of antibodies, such as those described, above, in Section 5.3,  
useful in the present invention may be used to quantitatively or qualitatively detect the  
presence of HGPRBMY1 or HGPRBMY2 gene products or conserved variants of the  
polypeptides or peptides. This can be accomplished, for example, by  
15 immunofluorescence techniques employing a fluorescently labeled antibody (see below,  
this Section) coupled with light microscopic, flow cytometric, or fluorimetric detection.  
Such techniques are especially preferred if such HGPRBMY1 or HGPRBMY2 gene  
products are expressed on the cell surface.

The antibodies (or fragments thereof) or agonist or antagonist fusion or  
conjugated polypeptides useful in the present invention may, additionally, be employed  
histologically, as in immunofluorescence, immunoelectron microscopy or non-immuno  
20 assays, for *in situ* detection of HGPRBMY1 or HGPRBMY2 gene products or conserved  
variants of the polypeptides or peptides, or for agonist or antagonist binding (in the case  
of labeled agonist or antagonist fusion polypeptide).

*In situ* detection may be accomplished by removing a histological specimen from  
25 a patient, and applying thereto a labeled antibody or fusion polypeptide of the present  
invention. The antibody (or fragment) or fusion polypeptide is preferably applied by  
overlaying the labeled antibody (or fragment) onto a biological sample. Through the use  
of such a procedure, it is possible to determine not only the presence of the HGPRBMY1  
or HGPRBMY2 gene product, or conserved variants of the polypeptides or peptides, or  
30 agonist or antagonist binding, but also its distribution in the examined tissue. Using the  
present invention, those of ordinary skill will readily perceive that any of a wide variety  
of histological methods (such as staining procedures) can be modified in order to achieve  
such *in situ* detection.

35 Immunoassays and non-immunoassays for HGPRBMY1 or HGPRBMY2 gene  
products or conserved variants of the polypeptides or peptides will typically comprise  
incubating a sample, such as a biological fluid, a tissue extract, freshly harvested cells,  
or lysates of cells which have been incubated in cell culture, in the presence of a

detectably labeled antibody capable of identifying HGPRBMY1 or HGPRBMY2 gene  
5 products or conserved variants of the polypeptides or peptides, and detecting the bound  
antibody by any of a number of techniques well-known in the art.

The biological sample may be brought in contact with and immobilized onto a  
solid phase support or carrier such as nitrocellulose, or other solid support which is  
10 capable of immobilizing cells, cell particles or soluble polypeptides. The support may  
then be washed with suitable buffers followed by treatment with the detectably labeled  
HGPRBMY1 or HGPRBMY2 antibody or agonist or antagonist fusion polypeptide. The  
solid phase support may then be washed with the buffer a second time to remove  
15 unbound antibody or fusion polypeptide. The amount of bound label on solid support may  
then be detected by conventional means.

By "solid phase support or carrier" is intended any support capable of binding an  
antigen or an antibody. Well-known supports or carriers include glass, polystyrene,  
polypropylene, polyethylene, dextran, nylon, amyloses, natural and modified celluloses,  
20 polyacrylamides, gabbros, and magnetite. The nature of the carrier can be either soluble  
to some extent or insoluble for the purposes of the present invention. The support  
material may have virtually any possible structural configuration so long as the coupled  
molecule is capable of binding to an antigen or antibody. Thus, the support configuration  
may be spherical, as in a bead, or cylindrical, as in the inside surface of a test tube, or the  
25 external surface of a rod. Alternatively, the surface may be flat such as a sheet, test strip,  
etc. Preferred supports include polystyrene beads. Those skilled in the art will know many  
other suitable carriers for binding antibody or antigen, or will be able to ascertain the  
same by use of routine experimentation. The binding activity of a given lot of  
30 HGPRBMY1 or HGPRBMY2 antibody or agonist or antagonist fusion polypeptide may  
be determined according to well known methods.

Those skilled in the art will be able to determine optimal assay conditions for  
each determination by employing routine experimentation.

With respect to antibodies, one of the ways in which the HGPRBMY1 or  
35 HGPRBMY2 antibody can be detectably labeled is by linking the same to an enzyme and  
used in an enzyme immunoassay (EIA) (Voller, A., "The Enzyme Linked Immunosorbent  
Assay (ELISA)", 1978, Diagnostic Horizons 2:1-7, Microbiological Associates Quarterly  
Publication, Walkersville, Md.); Voller, A. et al., 1978, J. Clin. Pathol. 31:507-520;

5      Butler, J. E., 1981, *Meth. Enzymol.* 73:482-523; Maggio, E. (ed.), 1980, *Enzyme Immunoassay*, CRC Press, Boca Raton, Fla.; Ishikawa, E. et al., (eds.), 1981, *Enzyme Immunoassay*, Kgaku Shoin, Tokyo).

10     The enzyme which is bound to the antibody will react with an appropriate substrate, preferably a chromogenic substrate, in such a manner as to produce a chemical moiety which can be detected, for example, by spectrophotometric, fluorimetric or by visual means. Enzymes which can be used to detectably label the antibody include, but are not limited to, malate dehydrogenase, staphylococcal nuclease, delta-5-steroid isomerase, yeast alcohol dehydrogenase, alphaglycerophosphate, dehydrogenase, triose phosphate isomerase, horseradish peroxidase, alkaline phosphatase, asparaginase, glucose 15 oxidase,  $\beta$ -galactosidase, ribonuclease, urease, catalase, glucose-6-phosphate dehydrogenase, glucoamylase and acetylcholinesterase. The detection can be accomplished by calorimetric methods which employ a chromogenic substrate for the enzyme. Detection may also be accomplished by visual comparison of the extent of enzymatic reaction of a substrate in comparison with similarly prepared standards.

20     Detection may also be accomplished using any of a variety of other immunoassays. For example, by radioactively labeling the antibodies or antibody fragments, it is possible to detect HGPRBMY1 or HGPRBMY2 through the use of a radioimmunoassay (RIA) (see, for example, Weintraub, B., *Principles of 25 Radioimmunoassays*, Seventh Training Course on Radioligand Assay Techniques, The Endocrine Society, March, 1986, which is incorporated by reference herein). The radioactive isotope can be detected by such means as the use of a gamma counter or a scintillation counter or by autoradiography.

30     It is also possible to label the antibody with a fluorescent compound. When the fluorescently labeled antibody is exposed to light of the proper wave length, its presence can then be detected due to fluorescence. Among the most commonly used fluorescent labeling compounds are fluorescein isothiocyanate, rhodamine, phycoerythrin, phycocyanin, allophycocyanin, o-phthaldehyde and fluorescamine.

35     The antibody can also be detectably labeled using fluorescence emitting metals such as  $^{152}\text{Eu}$ , or others of the lanthanide series. These metals can be attached to the antibody using such metal chelating groups as diethylenetriaminepentacetic acid (DTPA) or ethylenediaminetetraacetic acid (EDTA).

The antibody also can be detectably labeled by coupling it to a chemiluminescent compound. The presence of the chemiluminescent-tagged antibody is then determined by detecting the presence of luminescence that arises during the course of a chemical reaction. Examples of particularly useful chemiluminescent labeling compounds are luminol, isoluminol, theromatic acridinium ester, imidazole, acridinium salt and oxalate ester.

10

Likewise, a bioluminescent compound may be used to label the antibody of the present invention. Bioluminescence is a type of chemiluminescence found in biological systems in, which a catalytic polypeptide increases the efficiency of the chemiluminescent reaction. The presence of a bioluminescent polypeptide is determined by detecting the presence of luminescence. Important bioluminescent compounds for purposes of labeling are luciferin, luciferase and aequorin.

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5 **5.5. Screening Assays for Compounds that Modulate HGPRBMY1 and HGPRBMY2**

The following assays are designed to identify compounds that interact with (e.g., bind to) HGPRBMY1 or HGPRBMY2 (including, but not limited to the ECD or CD of HGPRBMY1 or HGPRBMY2), compounds that interact with (e.g., bind to) intracellular polypeptides that interact with HGPRBMY1 or HGPRBMY2 (including, but not limited to, the TM and CD of HGPRBMY1 or HGPRBMY2), compounds that interfere with the interaction of HGPRBMY1 or HGPRBMY2 with transmembrane or intracellular polypeptides involved in HGPRBMY1 or HGPRBMY2-mediated signal transduction, 15 and to compounds which modulate the activity of HGPRBMY1 or HGPRBMY2 gene (i.e., modulate the level of HGPRBMY1 or HGPRBMY2 gene expression) or modulate the level of HGPRBMY1 or HGPRBMY2. Assays may additionally be utilized which identify compounds which bind to HGPRBMY1 or HGPRBMY2 gene regulatory sequences (e.g., promoter sequences) and which may modulate HGPRBMY1 or 20 HGPRBMY2 gene expression. See e.g., Platt, K. A., 1994, *J. Biol. Chem.* 269:28558-28562, which is incorporated herein by reference in its entirety.

The compounds which may be screened in accordance with the invention include, but are not limited to peptides, antibodies and fragments thereof, and other organic compounds (e.g., peptidomimetics) that bind to the ECD of the HGPRBMY1 or HGPRBMY2 and either mimic the activity triggered by the natural ligand (i.e., agonists) or inhibit the activity triggered by the natural ligand (i.e., antagonists); as well as peptides, antibodies or fragments thereof, and other organic compounds that mimic the ECD of the HGPRBMY1 or HGPRBMY2 (or a portion thereof) and bind to and 30 “neutralize” natural ligand.

Such compounds may include, but are not limited to, peptides such as, for example, soluble peptides, including but not limited to members of random peptide libraries; (see, e.g., Lam, K. S. et al., 1991, *Nature* 354:82-84; Houghten, R. et al., 1991, *Nature* 354:84-86), and combinatorial chemistry-derived molecular library made of D- 35 and/or L- configuration amino acids, phosphopeptides (including, but not limited to, members of random or partially degenerate, directed phosphopeptide libraries; see, e.g., Songyang, Z. et al., 1993, *Cell* 72:767-778), antibodies (including, but not limited to,

5 polyclonal, monoclonal, humanized, anti-idiotypic, chimeric or single chain antibodies, and FAb, F(ab')<sub>2</sub> and FAb expression library fragments, and epitope-binding fragments thereof), and small organic or inorganic molecules.

10 Other compounds which can be screened in accordance with the invention include but are not limited to small organic molecules which may gain entry into an appropriate cell (e.g., in the bone marrow or spleen) and affect the expression of the HGPRBMY1 gene or some other gene involved in the HGPRBMY1 signal transduction pathway (e.g., by interacting with the regulatory region or transcription factors involved in gene expression); or such compounds that affect the activity of the HGPRBMY1 (e.g., by inhibiting or enhancing the enzymatic activity of the CD) or the activity of some other 15 intracellular factor involved in the HGPRBMY1 signal transduction pathway, such as, for example, gp130.

20 Other compounds which can be screened in accordance with the invention include but are not limited to small organic molecules which may gain entry into an appropriate cell (e.g., in the heart) and affect the expression of the HGPRBMY2 gene or some other gene involved in the HGPRBMY2 signal transduction pathway (e.g., by interacting with the regulatory region or transcription factors involved in gene expression); or such compounds that affect the activity of the HGPRBMY2 (e.g., by 25 inhibiting or enhancing the enzymatic activity of the CD) or the activity of some other intracellular factor involved in the HGPRBMY2 signal transduction pathway, such as, for example, gp130.

30 Computer modelling and searching technologies permit identification of compounds, or the improvement of already identified compounds, that can modulate HGPRBMY1 or HGPRBMY2 expression or activity. Having identified such a compound or composition, the active sites or regions are identified. Such active sites might typically 35 be ligand binding sites, such as the interaction domains of agonist or antagonist with HGPRBMY1 or HGPRBMY2 itself. The active site can be identified using methods known in the art including, for example, from the amino acid sequences of peptides, from the nucleotide sequences of nucleic acids, or from study of complexes of the relevant compound or composition with its natural ligand. In the latter case, chemical or X-ray crystallographic methods can be used to find the active site by finding where on the factor the complexed ligand is found. Next, the three dimensional geometric structure of the

active site is determined. This can be done by known methods, including X-ray 5 crystallography, which can determine a complete molecular structure. On the other hand, solid or liquid phase NMR can be used to determine certain intra-molecular distances. Any other experimental method of structure determination can be used to obtain partial 10 or complete geometric structures. The geometric structures may be measured with a complexed ligand, natural or artificial, which may increase the accuracy of the active site structure determined.

If an incomplete or insufficiently accurate structure is determined, the methods of computer based numerical modelling can be used to complete the structure or improve 15 its accuracy. Any recognized modelling method may be used, including parameterized models specific to particular biopolymers such as polypeptides or nucleic acids, molecular dynamics models based on computing molecular motions, statistical mechanics models based on thermal ensembles, or combined models. For most types of models, standard molecular force fields, representing the forces between constituent atoms and groups, are necessary, and can be selected from force fields known in physical chemistry. 20 The incomplete or less accurate experimental structures can serve as constraints on the complete and more accurate structures computed by these modeling methods.

Finally, having determined the structure of the active site, either experimentally, by modeling, or by a combination, candidate modulating compounds can be identified 25 by searching databases containing compounds along with information on their molecular structure. Such a search seeks compounds having structures that match the determined active site structure and that interact with the groups defining the active site. Such a search can be manual, but is preferably computer assisted. These compounds found from this search are potential HGPRBMY1 or HGPRBMY2 modulating compounds.

30 Alternatively, these methods can be used to identify improved modulating compounds from an already known modulating compound or ligand. The composition of the known compound can be modified and the structural effects of modification can be determined using the experimental and computer modelling methods described above applied to the new composition. The altered structure is then compared to the active site 35 structure of the compound to determine if an improved fit or interaction results. In this manner systematic variations in composition, such as by varying side groups, can be

quickly evaluated to obtain modified modulating compounds or ligands of improved  
5 specificity or activity.

Further experimental and computer modeling methods useful to identify  
modulating compounds based upon identification of the active sites of agonist or  
antagonist, HGPRBMY1 or HGPRBMY2, and related transduction and transcription  
factors will be apparent to those of skill in the art.  
10

Examples of molecular modelling systems are the CHARMM and QUANTA  
programs (Polygen Corporation, Waltham, Mass.). CHARMM performs the energy  
minimization and molecular dynamics functions. QUANTA performs the construction,  
graphic modelling and analysis of molecular structure. QUANTA allows interactive  
15 construction, modification, visualization, and analysis of the behavior of molecules with  
each other.

A number of articles review computer modeling of drugs interactive with  
specific-polypeptides, such as Rotivinen, et al., 1988, Acta Pharmaceutical Fennica  
97:159-166; Ripka, New Scientist 54-57 (Jun. 16, 1988); McKinlay and Rossmann, 1989,  
20 Annu. Rev. Pharmacol. Toxicol. 29:111-122; Perry and Davies, OSAR: Quantitative  
Structure-Activity Relationships in Drug Design pp. 189-193 (Alan R. Liss, Inc. 1989);  
Lewis and Dean, 1989 Proc. R. Soc. Lond. 236:125-140 and 141-162; and, with respect  
to a model receptor for nucleic acid components, Askew, et al., 1989, J. Am. Chem. Soc.  
25 111:1082-1090. Other computer programs that screen and graphically depict chemicals  
are available from companies such as BioDesign, Inc. (Pasadena, Calif.), Allelix, Inc.  
(Mississauga, Ontario, Canada), and Hypercube, Inc. (Cambridge, Ontario). Although  
these are primarily designed for application to drugs specific to particular polypeptides,  
they can be adapted to design of drugs specific to regions of DNA or RNA, once that  
30 region is identified.

Although described above with reference to design and generation of compounds  
which could alter binding, one could also screen libraries of known compounds,  
including natural products or synthetic chemicals, and biologically active materials,  
including polypeptides, for compounds which are inhibitors or activators.  
35

Compounds identified via assays such as those described herein may be useful,  
for example, in elaborating the biological function of the HGPRBMY1 gene product, and  
for ameliorating immune disorders. Assays for testing the effectiveness of compounds,

identified by, for example, techniques such as those described in Section 5.5.1 through 5 5.5.3, are discussed, below, in Section 5.5.4.

Compounds identified via assays such as those described herein may be useful, for example, in elaborating the biological function of the HGPRBMY2 gene product, and for ameliorating cardiovascular disorders. Assays for testing the effectiveness of 10 compounds, identified by, for example, techniques such as those described in Section 5.5.1 through 5.5.3, are discussed, below, in Section 5.5.4.

The human HGPRBMY1 or HGPRBMY2 polypeptides and/or peptides of the present invention, or immunogenic fragments or oligopeptides thereof, can be used for screening therapeutic drugs or compounds in a variety of drug screening techniques. The 15 fragment employed in such a screening assay may be free in solution, affixed to a solid support, borne on a cell surface, or located intracellularly. The reduction or abolition of activity of the formation of binding complexes between the ion channel protein and the agent being tested can be measured. Thus, the present invention provides a method for screening or assessing a plurality of compounds for their specific binding affinity with 20 a HGPRBMY1 or HGPRBMY2 polypeptide, or a bindable peptide fragment, of this invention, comprising providing a plurality of compounds, combining the HGPRBMY1 or HGPRBMY2 polypeptide, or a bindable peptide fragment, with each of a plurality of compounds for a time sufficient to allow binding under suitable conditions and detecting 25 binding of the HGPRBMY1 or HGPRBMY2 polypeptide or peptide to each of the plurality of test compounds, thereby identifying the compounds that specifically bind to the HGPRBMY1 or HGPRBMY2 polypeptide or peptide.

Methods of identifying compounds that modulate the activity of the novel human HGPRBMY1 or HGPRBMY2 polypeptides and/or peptides are provided by the present 30 invention and comprise combining a potential or candidate compound or drug modulator of G-protein coupled receptor biological activity with an HGPRBMY1 or HGPRBMY2 polypeptide or peptide, for example, the HGPRBMY1 or HGPRBMY2 amino acid sequence as set forth in SEQ ID NO:2 or SEQ ID NO:14, and measuring an effect of the candidate compound or drug modulator on the biological activity of the HGPRBMY1 or 35 HGPRBMY2 polypeptide or peptide. Such measurable effects include, for example, physical binding interaction; the ability to cleave a suitable G-protein coupled receptor substrate; effects on native and cloned HGPRBMY1 or HGPRBMY2-expressing cell

line; and effects of modulators or other G-protein coupled receptor-mediated  
5 physiological measures.

Another method of identifying compounds that modulate the biological activity  
of the novel HGPRBMY1 or HGPRBMY2 polypeptides of the present invention  
comprises combining a potential or candidate compound or drug modulator of a G-  
10 protein coupled receptor biological activity with a host cell that expresses the  
HGPRBMY1 or HGPRBMY2 polypeptide and measuring an effect of the candidate  
compound or drug modulator on the biological activity of the HGPRBMY1 or  
HGPRBMY2 polypeptide. The host cell can also be capable of being induced to express  
the HGPRBMY1 or HGPRBMY2 polypeptide, e.g., via inducible expression.  
15 Physiological effects of a given modulator candidate on the HGPRBMY1 or  
HGPRBMY2 polypeptide can also be measured. Thus, cellular assays for particular G-  
protein coupled receptor modulators may be either direct measurement or quantification  
of the physical biological activity of the HGPRBMY1 or HGPRBMY2 polypeptide, or  
they may be measurement or quantification of a physiological effect. Such methods  
20 preferably employ a HGPRBMY1 or HGPRBMY2 polypeptide as described herein, or  
an overexpressed recombinant HGPRBMY1 or HGPRBMY2 polypeptide in suitable host  
cells containing an expression vector as described herein, wherein the HGPRBMY1 or  
HGPRBMY2 polypeptide is expressed, overexpressed, or undergoes upregulated  
25 expression.

Another aspect of the present invention embraces a method of screening for a  
compound that is capable of modulating the biological activity of a HGPRBMY1 or  
HGPRBMY2 polypeptide, comprising providing a host cell containing an expression  
vector harboring a nucleic acid sequence encoding a HGPRBMY1 or HGPRBMY2  
30 polypeptide, or a functional peptide or portion thereof (e.g., SEQ ID NOS:2); determining  
the biological activity of the expressed HGPRBMY1 or HGPRBMY2 polypeptide in the  
absence of a modulator compound; contacting the cell with the modulator compound and  
determining the biological activity of the expressed HGPRBMY1 or HGPRBMY2  
35 polypeptide in the presence of the modulator compound. In such a method, a difference  
between the activity of the HGPRBMY1 or HGPRBMY2 polypeptide in the presence of  
the modulator compound and in the absence of the modulator compound indicates a  
modulating effect of the compound.

Essentially any chemical compound can be employed as a potential modulator or 5 ligand in the assays according to the present invention. Compounds tested as G-protein coupled receptor modulators can be any small chemical compound, or biological entity (e.g., protein, sugar, nucleic acid, lipid). Test compounds will typically be small chemical 10 molecules and peptides. Generally, the compounds used as potential modulators can be dissolved in aqueous or organic (e.g., DMSO-based) solutions. The assays are designed 15 to screen large chemical libraries by automating the assay steps and providing compounds from any convenient source. Assays are typically run in parallel, for example, in microtiter formats on microtiter plates in robotic assays. There are many suppliers of chemical compounds, including Sigma (St. Louis, MO), Aldrich (St. Louis, MO), Sigma- 20 Aldrich (St. Louis, MO), Fluka Chemika-Biochemica Analytika (Buchs, Switzerland), for example. Also, compounds may be synthesized by methods known in the art.

High throughput screening methodologies are particularly envisioned for the detection of modulators of the novel HGPRBMY1 or HGPRBMY2 polynucleotides and 20 polypeptides described herein. Such high throughput screening methods typically involve 25 providing a combinatorial chemical or peptide library containing a large number of potential therapeutic compounds (e.g., ligand or modulator compounds). Such combinatorial chemical libraries or ligand libraries are then screened in one or more assays to identify those library members (e.g., particular chemical species or subclasses) that display a desired characteristic activity. The compounds so identified can serve as conventional lead compounds, or can themselves be used as potential or actual therapeutics.

A combinatorial chemical library is a collection of diverse chemical compounds generated either by chemical synthesis or biological synthesis, by combining a number 30 of chemical building blocks (i.e., reagents such as amino acids). As an example, a linear combinatorial library, e.g., a polypeptide or peptide library, is formed by combining a set of chemical building blocks in every possible way for a given compound length (i.e., the 35 number of amino acids in a polypeptide or peptide compound). Millions of chemical compounds can be synthesized through such combinatorial mixing of chemical building blocks.

The preparation and screening of combinatorial chemical libraries is well known to those having skill in the pertinent art. Combinatorial libraries include, without

limitation, peptide libraries (e.g. U.S. Patent No. 5,010,175; Furka, 1991, *Int. J. Pept. Prot. Res.*, 37:487-493; and Houghton et al., 1991, *Nature*, 354:84-88). Other chemistries for generating chemical diversity libraries can also be used. Nonlimiting examples of chemical diversity library chemistries include, peptides (PCT Publication No. WO 91/019735), encoded peptides (PCT Publication No. WO 93/20242), random bio-oligomers (PCT Publication No. WO 92/00091), benzodiazepines (U.S. Patent No. 5,288,514), diversomers such as hydantoins, benzodiazepines and dipeptides (Hobbs et al., 1993, *Proc. Natl. Acad. Sci. USA*, 90:6909-6913), vinylogous polypeptides (Hagihara et al., 1992, *J. Amer. Chem. Soc.*, 114:6568), nonpeptidal peptidomimetics with glucose scaffolding (Hirschmann et al., 1992, *J. Amer. Chem. Soc.*, 114:9217-9218), analogous organic synthesis of small compound libraries (Chen et al., 1994, *J. Amer. Chem. Soc.*, 116:2661), oligocarbamates (Cho et al., 1993, *Science*, 261:1303), and/or peptidyl phosphonates (Campbell et al., 1994, *J. Org. Chem.*, 59:658), nucleic acid libraries (see Ausubel, Berger and Sambrook, all *supra*), peptide nucleic acid libraries (U.S. Patent No. 5,539,083), antibody libraries (e.g., Vaughn et al., 1996, *Nature Biotechnology*, 14(3):309-314) and PCT/US96/10287), carbohydrate libraries (e.g., Liang et al., 1996, *Science*, 274:1520-1522) and U.S. Patent No. 5,593,853), small organic molecule libraries (e.g., benzodiazepines, Baum C&EN, Jan. 18, 1993, page 33; and U.S. Patent No. 5,288,514; isoprenoids, U.S. Patent No. 5,569,588; thiazolidinones and metathiazanones, U.S. Patent No. 5,549,974; pyrrolidines, U.S. Patent Nos. 5,525,735 and 5,519,134; morpholino compounds, U.S. Patent No. 5,506,337; and the like).

Devices for the preparation of combinatorial libraries are commercially available (e.g., 357 MPS, 390 MPS, Advanced Chem Tech, Louisville KY; Symphony, Rainin, Woburn, MA; 433A Applied Biosystems, Foster City, CA; 9050 Plus, Millipore, Bedford, MA). In addition, a large number of combinatorial libraries are commercially available (e.g., ComGenex, Princeton, NJ; Asinex, Moscow, Russia; Tripos, Inc., St. Louis, MO; ChemStar, Ltd., Moscow, Russia; 3D Pharmaceuticals, Exton, PA; Martek Biosciences, Columbia, MD, and the like).

In one embodiment, the invention provides solid phase based *in vitro* assays in a high throughput format, where the cell or tissue expressing an ion channel is attached to a solid phase substrate. In such high throughput assays, it is possible to screen up to several thousand different modulators or ligands in a single day. In particular, each well

of a microtiter plate can be used to perform a separate assay against a selected potential 5 modulator, or, if concentration or incubation time effects are to be observed, every 5-10 wells can test a single modulator. Thus, a single standard microtiter plate can assay about 96 modulators. If 1536 well plates are used, then a single plate can easily assay from about 100 to about 1500 different compounds. It is possible to assay several different 10 plates per day; thus, for example, assay screens for up to about 6,000-20,000 different compounds are possible using the described integrated systems.

In another of its aspects, the present invention encompasses screening and small molecule (e.g., drug) detection assays which involve the detection or identification of 15 small molecules that can bind to a given protein, i.e., a HGPRBMY1 or HGPRBMY2 polypeptide or peptide. Particularly preferred are assays suitable for high throughput screening methodologies.

In such binding-based detection, identification, or screening assays, a functional 20 assay is not typically required. All that is needed is a target protein, preferably substantially purified, and a library or panel of compounds (e.g., ligands, drugs, small molecules) or biological entities to be screened or assayed for binding to the protein target. Preferably, most small molecules that bind to the target protein will modulate activity in some manner, due to preferential, higher affinity binding to functional areas or sites on the protein.

An example of such an assay is the fluorescence based thermal shift assay (3-25 Dimensional Pharmaceuticals, Inc., 3DP, Exton, PA) as described in U.S. Patent Nos. 6,020,141 and 6,036,920 to Pantoliano et al.; see also, J. Zimmerman, 2000, *Gen. Eng. News*, 20(8)). The assay allows the detection of small molecules (e.g., drugs, ligands) that bind to expressed, and preferably purified, ion channel polypeptide based on affinity of 30 binding determinations by analyzing thermal unfolding curves of protein-drug or ligand complexes. The drugs or binding molecules determined by this technique can be further assayed, if desired, by methods, such as those described herein, to determine if the molecules affect or modulate function or activity of the target protein.

To purify a HGPRBMY1 or HGPRBMY2 polypeptide or peptide to measure a 35 biological binding or ligand binding activity, the source may be a whole cell lysate that can be prepared by successive freeze-thaw cycles (e.g., one to three) in the presence of standard protease inhibitors. The HGPRBMY1 or HGPRBMY2 polypeptide may be

5 partially or completely purified by standard protein purification methods, e.g., affinity chromatography using specific antibody described *infra*, or by ligands specific for an epitope tag engineered into the recombinant HGPRBMY1 or HGPRBMY2 polypeptide molecule, also as described herein. Binding activity can then be measured as described.

10 Compounds which are identified according to the methods provided herein, and which modulate or regulate the biological activity or physiology of the HGPRBMY1 or HGPRBMY2 polypeptides according to the present invention are a preferred embodiment of this invention. It is contemplated that such modulatory compounds may be employed in treatment and therapeutic methods for treating a condition that is mediated by the novel HGPRBMY1 or HGPRBMY2 polypeptides by administering to an individual in 15 need of such treatment a therapeutically effective amount of the compound identified by the methods described herein.

20 In addition, the present invention provides methods for treating an individual in need of such treatment for a disease, disorder, or condition that is mediated by the HGPRBMY1 or HGPRBMY2 polypeptides of the invention, comprising administering to the individual a therapeutically effective amount of the HGPRBMY1 or HGPRBMY2-modulating compound identified by a method provided herein.

25 **5.5.1. *In Vitro* Screening Assays for Compounds that Bind to HGPRBMY1 or HGPRBMY2**

30 *In vitro* systems may be designed to identify compounds capable of interacting with (e.g., binding to) HGPRBMY1 or HGPRBMY2 (including, but not limited to, the ECD or CD of HGPRBMY1 or HGPRBMY2). Compounds identified may be useful, for example, in modulating the activity of wild type and/or mutant HGPRBMY1 or HGPRBMY2 gene products; may be useful in elaborating the biological function of the HGPRBMY1 or HGPRBMY2; may be utilized in screens for identifying compounds that disrupt normal HGPRBMY1 or HGPRBMY2 interactions; or may in themselves disrupt such interactions.

35 The principle of the assays used to identify compounds that bind to the HGPRBMY1 or HGPRBMY2 involves preparing a reaction mixture of the HGPRBMY1 or HGPRBMY2 and the test compound under conditions and for a time sufficient to

allow the two components to interact and bind, thus forming a complex which can be  
5 removed and/or detected in the reaction mixture. The HGPRBMY1 or HGPRBMY2  
species used can vary depending upon the goal of the screening assay. For example,  
where agonists of the natural ligand are sought, the full length HGPRBMY1 or  
HGPRBMY2, or a soluble truncated HGPRBMY1 or HGPRBMY2, *e.g.*, in which the  
10 TM and/or CD is deleted from the molecule, a peptide corresponding to the ECD or a  
fusion polypeptide containing the HGPRBMY1 or HGPRBMY2 ECD fused to a  
polypeptide or peptide that affords advantages in the assay system (*e.g.*, labeling,  
isolation of the resulting complex, etc.) can be utilized. Where compounds that interact  
15 with the cytoplasmic domain are sought to be identified, peptides corresponding to the  
HGPRBMY1 or HGPRBMY2 CD and fusion polypeptides containing the HGPRBMY1  
or HGPRBMY2 CD can be used.

The screening assays can be conducted in a variety of ways. For example, one  
method to conduct such an assay would involve anchoring the HGPRBMY1 or  
HGPRBMY2 polypeptide, peptide or fusion polypeptide or the test substance onto a solid  
20 phase and detecting HGPRBMY1 or HGPRBMY2/test compound complexes anchored  
on the solid phase at the end of the reaction. In one embodiment of such a method, the  
HGPRBMY1 or HGPRBMY2 reactant may be anchored onto a solid surface, and the test  
compound, which is not anchored, may be labeled, either directly or indirectly.

25 In practice, microtiter plates may conveniently be utilized as the solid phase. The  
anchored component may be immobilized by non-covalent or covalent attachments.  
Non-covalent attachment may be accomplished by simply coating the solid surface with  
a solution of the polypeptide and drying. Alternatively, an immobilized antibody,  
preferably a monoclonal antibody, specific for the polypeptide to be immobilized may be  
30 used to anchor the polypeptide to the solid surface. The surfaces may be prepared in  
advance and stored.

35 In order to conduct the assay, the nonimmobilized component is added to the  
coated surface containing the anchored component. After the reaction is complete,  
unreacted components are removed (*e.g.*, by washing) under conditions such that any  
complexes formed will remain immobilized on the solid surface. The detection of  
complexes anchored on the solid surface can be accomplished in a number of ways.  
Where the previously nonimmobilized component is pre-labeled, the detection of label

immobilized on the surface indicates that complexes were formed. Where the previously 5 nonimmobilized component is not pre-labeled, an indirect label can be used to detect complexes anchored on the surface; *e.g.*, using a labeled antibody specific for the previously nonimmobilized component (the antibody, in turn, may be directly labeled or indirectly labeled with a labeled anti-Ig antibody).

10 Alternatively, a reaction can be conducted in a liquid phase, the reaction products separated from unreacted components, and complexes detected; *e.g.*, using an immobilized antibody specific for HGPRBMY1 or HGPRBMY2 polypeptide, peptide or fusion polypeptide or the test compound to anchor any complexes formed in solution, and a labeled antibody specific for the other component of the possible complex to detect 15 anchored complexes.

20 Alternatively, cell-based assays can be used to identify compounds that interact with HGPRBMY1 or HGPRBMY2. To this end, cell lines that express HGPRBMY1 or HGPRBMY2, or cell lines (*e.g.*, COS cells, CHO cells, fibroblasts, etc.) that have been genetically engineered to express HGPRBMY1 or HGPRBMY2 (*e.g.*, by transfection or 25 transduction of HGPRBMY1 or HGPRBMY2 DNA) can be used. Interaction of the test compound with, for example, the ECD of HGPRBMY1 or HGPRBMY2 expressed by the host cell can be determined by comparison or competition with native agonist or antagonist.

25

### 5.5.2. Assays for Polypeptides that Interact with the HGPRBMY1 or HGPRBMY2

Any method suitable for detecting polypeptide-polypeptide interactions may be employed for identifying transmembrane polypeptides or intracellular polypeptides that interact with HGPRBMY1 or HGPRBMY2. Among the traditional methods which may 30 be employed are co-immunoprecipitation, crosslinking and co-purification through gradients or chromatographic columns of cell lysates or polypeptides obtained from cell lysates and the HGPRBMY1 or HGPRBMY2 to identify polypeptides in the lysate that interact with the HGPRBMY1 or HGPRBMY2. For these assays, the HGPRBMY1 or 35 HGPRBMY2 component used can be a full length HGPRBMY1 or HGPRBMY2, a soluble derivative lacking the membrane-anchoring region (*e.g.*, a truncated HGPRBMY1 or HGPRBMY2 in which the TM is deleted resulting in a truncated molecule containing the ECD fused to the CD), a peptide corresponding to the CD or a fusion polypeptide

containing the CD of HGPRBMY1 or HGPRBMY2. Once isolated, such an intracellular  
5 polypeptide can be identified and can, in turn, be used, in conjunction with standard  
techniques, to identify polypeptides with which it interacts. For example, at least a  
portion of the amino acid sequence of an intracellular polypeptide which interacts with  
the HGPRBMY1 or HGPRBMY2 can be ascertained using techniques well known to  
those of skill in the art, such as via the Edman degradation technique (See, e.g.,  
10 Creighton, 1983, "Proteins: Structures and Molecular Principles", W.H. Freeman & Co.,  
N.Y., pp.34-49). The amino acid sequence obtained may be used as a guide for the  
generation of oligonucleotide mixtures that can be used to screen for gene sequences  
encoding such intracellular polypeptides. Screening may be accomplished, for example,  
15 by standard hybridization or PCR techniques. Techniques for the generation of  
oligonucleotide mixtures and the screening are well-known (See, e.g., Ausubel, *supra*,  
and PCR Protocols: A Guide to Methods and Applications, 1990, Innis, M. et al., eds.  
Academic Press, Inc., New York).

Additionally, methods may be employed which result in the simultaneous  
20 identification of genes which encode the transmembrane or intracellular polypeptides  
interacting with HGPRBMY1 or HGPRBMY2. These methods include, for example,  
probing expression, libraries, in a manner similar to the well known technique of  
antibody probing of λgt11 libraries, using labeled HGPRBMY1 or HGPRBMY2  
25 polypeptide, or an HGPRBMY1 or HGPRBMY2 polypeptide, peptide or fusion  
polypeptide, e.g., an HGPRBMY1 or HGPRBMY2 polypeptide or HGPRBMY1 or  
HGPRBMY2 domain fused to a marker (e.g., an enzyme, fluor, luminescent polypeptide,  
or dye), or an Ig-Fc domain.

One method which detects polypeptide interactions *in vivo*, the two-hybrid  
30 system, is described in detail for illustration only and not by way of limitation. One  
version of this system has been described (Chien et al., 1991, Proc. Natl. Acad. Sci. USA,  
88:9578-9582) and is commercially available from Clontech (Palo Alto, Calif.).

Briefly, utilizing such a system, plasmids are constructed that encode two hybrid  
35 polypeptides: one plasmid consists of nucleic acids encoding the DNA-binding domain  
of a transcription activator polypeptide fused to an HGPRBMY1 or HGPRBMY2 nucleic  
acid sequence encoding HGPRBMY1 or HGPRBMY2, an HGPRBMY1 or HGPRBMY2  
polypeptide, peptide or fusion polypeptide, and the other plasmid consists of nucleic

acids encoding the transcription activator polypeptide's activation domain fused to a  
5 cDNA encoding an unknown polypeptide which has been recombined into this plasmid  
as part of a cDNA library. The DNA-binding domain fusion plasmid and the cDNA  
library are transformed into a strain of the yeast *Saccharomyces cerevisiae* that contains  
a reporter gene (*e.g.*, HBS or lacZ) whose regulatory region contains the transcription  
activator's binding site. Either hybrid polypeptide alone cannot activate transcription of  
10 the reporter gene: the DNA-binding domain hybrid cannot because it does not provide  
activation function and the activation domain hybrid cannot because it cannot localize to  
the activator's binding sites. Interaction of the two hybrid polypeptides reconstitutes the  
functional activator polypeptide and results in expression of the reporter gene, which is  
15 detected by an assay for the reporter gene product.

The two-hybrid system or related methodology may be used to screen activation  
domain libraries for polypeptides that interact with the "bait" gene product. By way of  
example, and not by way of limitation, HGPRBMY1 or HGPRBMY2 may be used as the  
20 bait gene product. Total genomic or cDNA sequences are fused to the DNA encoding an  
activation domain. This library and a plasmid encoding a hybrid of a bait HGPRBMY1  
or HGPRBMY2 gene product fused to the DNA-binding domain are cotransformed into  
a yeast reporter strain, and the resulting transformants are screened for those that express  
the reporter gene. For example, and not by way of limitation, a bait HGPRBMY1 or  
25 HGPRBMY2 gene sequence, such as the open reading frame of HGPRBMY1 or  
HGPRBMY2 (or a domain of HGPRBMY1 or HGPRBMY2), as depicted in Figure 1 can  
be cloned into a vector such that it is translationally fused to the DNA encoding the  
DNA-binding domain of the GAL4 polypeptide. These colonies are purified and the  
library plasmids responsible for reporter gene expression are isolated. DNA sequencing  
30 is then used to identify the polypeptides encoded by the library plasmids.

A cDNA library of the cell line from which polypeptides that interact with bait  
HGPRBMY1 or HGPRBMY2 gene product are to be detected can be made using  
methods routinely practiced in the art. According to the particular system described  
35 herein, for example, the cDNA fragments can be inserted into a vector such that they are  
translationally fused to the transcriptional activation domain of GAL4. This library can  
be co-transformed along with the bait HGPRBMY1 or HGPRBMY2 gene-GAL4 fusion  
plasmid into a yeast strain which contains a lacZ gene driven by a promoter which

contains GAL4 activation sequence. A cDNA encoded polypeptide, fused to GAL4 transcriptional activation domain, that interacts with bait HGPRBMY1 or HGPRBMY2 gene product will reconstitute an active GAL4 polypeptide and thereby drive expression of the HIS3 gene. Colonies which express HIS3 can be detected by their growth on petri dishes containing semi-solid agar based media lacking histidine. The cDNA can then be purified from these strains, and used to produce and isolate the bait HGPRBMY1 or HGPRBMY2 gene-interacting polypeptide using techniques routinely practiced in the art.

10 Additional assays for identifying polypeptides that bind to and potentially modulate the HGPRBMY1 or HGPRBMY2 polypeptides are described elsewhere herein. More specifically, peptides have been identified that have been shown to bind to and 15 potentially modulate the HGPRBMY2 polypeptide.

#### 5.5.3. Assays for other Compounds

The macromolecules that interact with the HGPRBMY1 are referred to, for purposes of this discussion, as "binding partners". These binding partners are likely to be 20 involved in the HGPRBMY1 signal transduction pathway, and therefore, in the role of HGPRBMY1 in immune related regulation. Therefore, it is desirable to identify compounds that interfere with or disrupt the interaction of such binding partners with agonist or antagonist which may be useful in regulating the activity of the HGPRBMY1 25 and control immune disorders associated with HGPRBMY1 activity.

The macromolecules that interact with the HGPRBMY2 are referred to, for purposes of this discussion, as "binding partners". These binding partners are likely to be involved in the HGPRBMY2 signal transduction pathway, and therefore, in the role of HGPRBMY2 in cardiovascular regulation. Therefore, it is desirable to identify 30 compounds that interfere with or disrupt the interaction of such binding partners with agonist or antagonist which may be useful in regulating the activity of the HGPRBMY2 and control cardiovascular or neural disorders associated with HGPRBMY2 activity.

The basic principle of the assay systems used to identify compounds that interfere 35 with the interaction between the HGPRBMY1 or HGPRBMY2 and its binding partner or partners involves preparing a reaction mixture containing HGPRBMY1 or HGPRBMY2 polypeptide, peptide or fusion polypeptide as described in Sections 5.5.1 and 5.5.2 above, and the binding partner under conditions and for a time sufficient to

allow the two to interact and bind, thus forming a complex. In order to test a compound  
5 for inhibitory activity, the reaction mixture is prepared in the presence and absence of the  
test compound. The test compound may be initially included in the reaction mixture, or  
may be added at a time subsequent to the addition of the HGPRBMY1 or HGPRBMY2  
moiety and its binding partner. Control reaction mixtures are incubated without the test  
10 compound or with a placebo. The formation of any complexes between the HGPRBMY1  
or HGPRBMY2 moiety and the binding partner is then detected. The formation of a  
complex in the control reaction, but not in the reaction mixture containing the test  
compound, indicates that the compound interferes with the interaction of the  
HGPRBMY1 or HGPRBMY2 and the interactive binding partner. Additionally, complex  
15 formation within reaction mixtures containing the test compound and normal  
HGPRBMY1 or HGPRBMY2 polypeptide may also be compared to complex formation  
within reaction mixtures containing the test compound and a mutant HGPRBMY1 or  
HGPRBMY2. This comparison may be important in those cases wherein it is desirable  
20 to identify compounds that disrupt interactions of mutant but not normal HGPRBMY1  
or HGPRBMY2.

The assay for compounds that interfere with the interaction of the HGPRBMY1  
or HGPRBMY2 and binding partners can be conducted in a heterogeneous or  
homogeneous format. Heterogeneous assays involve anchoring either the HGPRBMY1  
25 or HGPRBMY2 moiety product or the binding partner onto a solid phase and detecting  
complexes anchored on the solid phase at the end of the reaction. In homogeneous assays,  
the entire reaction is carried out in a liquid phase. In either approach, the order of addition  
of reactants can be varied to obtain different information about the compounds being  
tested. For example, test compounds that interfere with the interaction by competition can  
30 be identified by conducting the reaction in the presence of the test substance; *i.e.*, by  
adding the test substance to the reaction mixture prior to or simultaneously with the  
HGPRBMY1 or HGPRBMY2 moiety and interactive binding partner. Alternatively, test  
compounds that disrupt preformed complexes, *e.g.* compounds with higher binding  
35 constants that displace one of the components from the complex, can be tested by adding  
the test compound to the reaction mixture after complexes have been formed. The various  
formats are described briefly below.

5 In a heterogeneous assay system, either the HGPRBMY1 or HGPRBMY2 moiety  
or the interactive binding partner, is anchored onto a solid surface, while the  
non-anchored species is labeled, either directly or indirectly. In practice, microtiter plates  
are conveniently utilized. The anchored species may be immobilized by non-covalent or  
covalent attachments. Non-covalent attachment may be accomplished simply by coating  
the solid surface with a solution of the HGPRBMY1 or HGPRBMY2 gene product or  
10 binding partner and drying. Alternatively, an immobilized antibody specific for the  
species to be anchored may be used to anchor the species to the solid surface. The  
surfaces may be prepared in advance and stored.

15 In order to conduct the assay, the partner of the immobilized species is exposed  
to the coated surface with or without the test compound. After the reaction is complete,  
unreacted components are removed (e.g., by washing) and any complexes formed will  
remain immobilized on the solid surface. The detection of complexes anchored on the  
solid surface can be accomplished in a number of ways. Where the non-immobilized  
20 species is pre-labeled, the detection of label immobilized on the surface indicates that  
complexes were formed. Where the non-immobilized species is not pre-labeled, an  
indirect label can be used to detect complexes anchored on the surface; e.g., using a  
labeled antibody specific for the initially non-immobilized species (the antibody, in turn,  
may be directly labeled or indirectly labeled with a labeled anti-Ig antibody). Depending  
25 upon the order of addition of reaction components, test compounds which inhibit  
complex formation or which disrupt preformed complexes can be detected.

30 Alternatively, the reaction can be conducted in a liquid phase in the presence or  
absence of the test compound, the reaction products separated from unreacted  
components, and complexes detected; e.g., using an immobilized antibody specific for  
one of the binding components to anchor any complexes formed in solution, and a labeled  
35 antibody specific for the other partner to detect anchored complexes. Again, depending  
upon the order of addition of reactants to the liquid phase, test compounds which inhibit  
complex or which disrupt preformed complexes can be identified.

35 In an alternate embodiment of the invention, a homogeneous assay can be used.  
In this approach, a preformed complex of the HGPRBMY1 or HGPRBMY2 moiety and  
the interactive binding partner is prepared in which either the HGPRBMY1 or  
HGPRBMY2 or its binding partners is labeled, but the signal generated by the label is

quenched due to formation of the complex (see, *e.g.*, U.S. Pat. No. 4,109,496 by 5 Rubenstein which utilizes this approach for immunoassays). The addition of a test substance that competes with and displaces one of the species from the preformed complex will result in the generation of a signal above background. In this way, test substances which disrupt HGPRBMY1 or HGPRBMY2/intracellular binding partner interaction can be identified.

10 In a particular embodiment, an HGPRBMY1 or HGPRBMY2 fusion can be prepared for immobilization. For example, the HGPRBMY1 or HGPRBMY2 polypeptides or peptides, *e.g.*, corresponding to the CD, can be fused to a glutathione-S-transferase (GST) gene using a fusion vector, such as pGEX-5X-1, in such 15 a manner that its binding activity is maintained in the resulting fusion polypeptide. The interactive binding partner can be purified and used to raise a monoclonal antibody, using methods routinely practiced in the art and described above, in Section 5.3. This antibody can be labeled with the radioactive isotope  $^{125}\text{I}$ , for example, by methods routinely practiced in the art. In a heterogeneous assay, *e.g.*, the GST-HGPRBMY1 or 20 HGPRBMY2 fusion polypeptide can be anchored to glutathione-agarose beads. The interactive binding partner can then be added in the presence or absence of the test compound in a manner that allows interaction and binding to occur. At the end of the reaction period, unbound material can be washed away, and the labeled monoclonal 25 antibody can be added to the system and allowed to bind to the complexed components. The interaction between the HGPRBMY1 or HGPRBMY2 gene product and the interactive binding partner can be detected by measuring the amount of radioactivity that remains associated with the glutathione-agarose beads. A successful inhibition of the interaction by the test compound will result in a decrease in measured radioactivity.

30 Alternatively, the GST-HGPRBMY1 or HGPRBMY2 fusion polypeptide and the interactive binding partner can be mixed together in liquid in the absence of the solid glutathione-agarose beads. The test compound can be added either during or after the species are allowed to interact. This mixture can then be added to the glutathione-agarose 35 beads and unbound material is washed away. Again the extent of inhibition of the HGPRBMY1 or HGPRBMY2/binding partner interaction can be detected by adding the labeled antibody and measuring the radioactivity associated with the beads.

In another embodiment of the invention, these same techniques can be employed 5 using polypeptides or peptides that correspond to the binding domains of the HGPRBMY1 or HGPRBMY2 and/or the interactive or binding partner (in cases where the binding partner is a polypeptide), in place of one or both of the full length polypeptides. Any number of methods routinely practiced in the art can be used to 10 identify and isolate the binding sites. These methods include, but are not limited to, mutagenesis of the gene encoding one of the polypeptides and screening for disruption of binding in a co-immunoprecipitation assay. compensating mutations in the gene encoding the second species in the complex can then be selected. Sequence analysis of the genes encoding the respective polypeptides will reveal the mutations that correspond 15 to the region of the polypeptide involved in interactive binding.

Alternatively, one polypeptide can be anchored to a solid surface using methods described above, and allowed to interact with and bind to its labeled binding partner, which has been treated with a proteolytic enzyme, such as trypsin. After washing, a short, labeled peptide comprising the binding domain may remain associated with the solid 20 material, which can be isolated and identified by amino acid sequencing. Also, once the gene coding for the intracellular binding partner is obtained, short gene segments can be engineered to express polypeptides or peptides of the invention, which can then be tested for binding activity and purified or synthesized.

For example, and not by way of limitation, an HGPRBMY1 or HGPRBMY2 25 gene product can be anchored to a solid material by making a GST-HGPRBMY1 or HGPRBMY2 fusion polypeptide and allowing it to bind to glutathione agarose beads. The interactive binding partner can be labeled with a radioactive isotope, such as <sup>35</sup>S, and cleaved with a proteolytic enzyme such as trypsin. Cleavage products can then be added 30 to the anchored GST-HGPRBMY1 or HGPRBMY2 fusion polypeptide and allowed to bind. After washing away unbound peptides, labeled bound material, representing the intracellular binding partner binding domain, can be eluted, purified, and analyzed for amino acid sequence by well-known methods. Peptides so identified can be produced 35 synthetically or fused to appropriate facilitative polypeptides using recombinant DNA technology.

#### **5.5.4. Assays for Identification of Compounds that Ameliorate Immune Disorders**

Compounds, including but not limited to binding compounds identified via assay techniques such as those described, above, in Sections 5.5.1 through 5.5.3, can be tested for the ability to ameliorate immune related disorder symptoms, including immunodeficiency. The assays described above can identify compounds which affect HGPRBMY1 activity (*e.g.*, compounds that bind to the HGPRBMY1, inhibit binding of the natural ligand, and either activate signal transduction (agonists) or block activation (antagonists), and compounds that bind to the natural ligand of the HGPRBMY1 and neutralize ligand activity); or compounds that affect HGPRBMY1 gene activity (by affecting HGPRBMY1 gene expression, including molecules, *e.g.*, polypeptides or small organic molecules, that affect or interfere with splicing events so that expression of the full length or the truncated form of the HGPRBMY1 can be modulated). However, it should be noted that the assays described can also identify compounds that modulate HGPRBMY1 signal transduction (*e.g.*, compounds which affect downstream signaling events, such as inhibitors or enhancers of tyrosine kinase or phosphatase activities which participate in transducing the signal activated by agonist or antagonist binding to the HGPRBMY1). The identification and use of such compounds which affect another step in the HGPRBMY1 signal transduction pathway in which the HGPRBMY1 gene and/or HGPRBMY1 gene product is involved and, by affecting this same pathway may modulate the effect of HGPRBMY1 on the development of immune disorders are within the scope of the invention. Such compounds can be used as part of a therapeutic method for the treatment of immune disorders.

The invention features cell-based and animal model-based assays for the identification of compounds exhibiting such an ability to ameliorate immune related disorder symptoms. Such cell-based assay systems can also be used as a standard to assay for purity and potency of the natural ligand, agonist or antagonist, including recombinantly or synthetically produced agonist or antagonist and agonist or antagonist mutants.

Cell-based systems can be used to identify compounds which may act to ameliorate immune related disorder symptoms. Such cell systems can include, for example, recombinant or non-recombinant cells, such as cell lines, which express the HGPRBMY1 gene. For example bone marrow or spleen cells, or cell lines derived from bone marrow or spleen can be used. In addition, expression host cells (*e.g.*, COS cells,

CHO cells, fibroblasts) genetically engineered to express a functional HGPRBMY1 and 5 to respond to activation by the natural agonist or antagonist ligand, *e.g.*, as measured by a chemical or phenotypic change, induction of another host cell gene, change in ion flux (*e.g.*,  $\text{Ca}^{++}$ ), tyrosine phosphorylation of host cell polypeptides, etc., can be used as an end point in the assay.

10 In utilizing such cell systems, cells may be exposed to a compound suspected of exhibiting an ability to ameliorate immune related disorder symptoms, at a sufficient concentration and for a time sufficient to elicit such an amelioration of immune related disorder symptoms in the exposed cells. After exposure, the cells can be assayed to measure alterations in the expression of the HGPRBMY1 gene, *e.g.*, by assaying cell 15 lysates for HGPRBMY1 mRNA transcripts (*e.g.*, by Northern analysis) or for HGPRBMY1 polypeptide expressed in the cell; compounds which regulate or modulate expression of the HGPRBMY1 gene are good candidates as therapeutics. Alternatively, the cells are examined to determine whether one or more immune related disorder-like 20 cellular phenotypes has been altered to resemble a more normal or more wild type, non-immune related disorder phenotype, or a phenotype more likely to produce a lower incidence or severity of disorder symptoms.

25 Still further, the expression and/or activity of components of the signal transduction pathway of which HGPRBMY1 is a part, or the activity of the HGPRBMY1 signal transduction pathway itself can be assayed. For example, after exposure, the cell lysates can be assayed for the presence of tyrosine phosphorylation of host cell polypeptides, as compared to lysates derived from unexposed control cells. The ability 30 of a test compound to inhibit tyrosine phosphorylation of host cell polypeptides in these assay systems indicates that the test compound inhibits signal transduction initiated by HGPRBMY1 activation. The cell lysates can be readily assayed using a Western blot format; *i.e.*, the host cell polypeptides are resolved by gel electrophoresis, transferred and probed using a anti-phosphorylated amino acid detection antibody (*e.g.*, an anti-phosphotyrosine antibody labeled with a signal generating compound, such as 35 radiolabel, fluor, enzyme, etc.) (See, *e.g.*, Glenney et al., 1988, *J. Immunol. Methods* 109:277-285; Frackelton et al., 1983, *Mol. Cell. Biol.* 3:1343-1352). Alternatively, an ELISA format could be used in which a particular host cell polypeptide involved in the HGPRBMY1 signal transduction pathway is immobilized using an anchoring antibody

specific for the target host cell polypeptide, and the presence or absence of 5 phosphorylated amino acid residues, for example on tyrosine, on the immobilized host cell polypeptide is detected using a labeled anti-phosphotyrosine antibody (See, King et al., 1993, *Life Sciences* 53:1465-1472). In yet another approach, ion flux, such as calcium ion flux, can be measured as an end point for HGPRBMY1 stimulated signal 10 transduction.

10 In addition, animal-based immune related disorder systems may for example be used to identify compounds capable of ameliorating immune related disorder-like symptoms. Such animal models may be used as test substrates for the identification of drugs, pharmaceuticals, therapies and interventions which may be effective in treating 15 such disorders. For example, animal models may be exposed to a compound, suspected of exhibiting an ability to ameliorate immune related disorder symptoms, at a sufficient concentration and for a time sufficient to elicit such an amelioration of immune related disorder symptoms in the exposed animals. The response of the animals to the exposure may be monitored by assessing the reversal of disorders associated with immune 20 disorders such as immunodeficiency. With regard to intervention, any treatments which reverse any aspect of immune related disorder-like symptoms should be considered as candidates for human immune related disorder therapeutic intervention. Dosages of test agents may be determined by deriving dose-response curves, as discussed in Section 25 5.7.1, below.

#### **5.5.4b. Assays for Identification of Compounds that Ameliorate Cardiovascular Disorders**

Compounds, including but not limited to binding compounds identified via assay 30 techniques such as those described, above, in Sections 5.5.1 through 5.5.3, can be tested for the ability to ameliorate cardiovascular disorder symptoms, including congestive heart failure. The assays described above can identify compounds which affect HGPRBMY2 activity (*e.g.*, compounds that bind to the HGPRBMY2, inhibit binding of the natural 35 ligand, and either activate signal transduction (agonists) or block activation (antagonists), and compounds that bind to the natural ligand of the HGPRBMY2 and neutralize ligand activity); or compounds that affect HGPRBMY2 gene activity (by affecting HGPRBMY2 gene expression, including molecules, *e.g.*, polypeptides or small organic molecules, that

5 affect or interfere with splicing events so that expression of the full length or the truncated form of the HGPRBMY2 can be modulated). However, it should be noted that the assays described can also identify compounds that modulate HGPRBMY2 signal transduction (e.g., compounds which affect downstream signalling events, such as inhibitors or enhancers of tyrosine kinase or phosphatase activities which participate in transducing the signal activated by agonist or antagonist binding to the HGPRBMY2).  
10 The identification and use of such compounds which affect another step in the HGPRBMY2 signal transduction pathway in which the HGPRBMY2 gene and/or HGPRBMY2 gene product is involved and, by affecting this same pathway may modulate the effect of HGPRBMY2 on the development of cardiovascular disorders are  
15 within the scope of the invention. Such compounds can be used as part of a therapeutic method for the treatment of cardiovascular disorders.

20 The invention encompasses cell-based and animal model-based assays for the identification of compounds exhibiting such an ability to ameliorate cardiovascular disorder symptoms. Such cell-based assay systems can also be used as a standard to assay for purity and potency of the natural ligand, agonist or antagonist, including recombinantly or synthetically produced agonist or antagonist and agonist or antagonist mutants.

25 Cell-based systems can be used to identify compounds which may act to ameliorate cardiovascular disorder symptoms. Such cell systems can include, for example, recombinant or non-recombinant cells, such as cell lines, which express the HGPRBMY2 gene. For example heart cells, or cell lines derived from heart can be used. In addition, expression host cells (e.g., COS cells, CHO cells, fibroblasts) genetically engineered to express a functional HGPRBMY2 and to respond to activation by the  
30 natural agonist or antagonist ligand, e.g., as measured by a chemical or phenotypic change, induction of another host cell gene, change in ion flux (e.g.,  $\text{Ca}^{++}$ ), tyrosine phosphorylation of host cell polypeptides, etc., can be used as an end point in the assay.

35 In utilizing such cell systems, cells may be exposed to a compound suspected of exhibiting an ability to ameliorate cardiovascular disorder symptoms, at a sufficient concentration and for a time sufficient to elicit such an amelioration of cardiovascular disorder symptoms in the exposed cells. After exposure, the cells can be assayed to measure alterations in the expression of the HGPRBMY2 gene, e.g., by assaying cell

lysates for HGPRBMY2 mRNA transcripts (e.g., by Northern analysis) or for 5 HGPRBMY2 polypeptide expressed in the cell; compounds which regulate or modulate expression of the HGPRBMY2 gene are good candidates as therapeutics. Alternatively, the cells are examined to determine whether one or more cardiovascular disorder-like 10 cellular phenotypes has been altered to resemble a more normal or more wild type, non-cardiovascular disorder phenotype, or a phenotype more likely to produce a lower incidence or severity of disorder symptoms. Still further, the expression and/or activity 15 of components of the signal transduction pathway of which HGPRBMY2 is a part, or the activity of the HGPRBMY2 signal transduction pathway itself can be assayed. For example, after exposure, the cell lysates can be assayed for the presence of tyrosine phosphorylation of host cell polypeptides, as compared to lysates derived from unexposed 20 control cells. The ability of a test compound to inhibit tyrosine phosphorylation of host cell polypeptides in these assay systems indicates that the test compound inhibits signal transduction initiated by HGPRBMY2 activation. The cell lysates can be readily assayed using a Western blot format; i.e., the host cell polypeptides are resolved by gel 25 electrophoresis, transferred and probed using a anti-phosphotyrosine detection antibody (e.g., an anti-phosphotyrosine antibody labeled with a signal generating compound, such as radiolabel, fluor, enzyme, etc.) (See, e.g., Glenney et al., 1988, J. Immunol. Methods 109:277-285; Frackelton et al., 1983, Mol. Cell. Biol. 3:1343-1352). Alternatively, an 30 ELISA format could be used in which a particular host cell polypeptide involved in the HGPRBMY2 signal transduction pathway is immobilized using an anchoring antibody specific for the target host cell polypeptide, and the presence or absence of phosphotyrosine on the immobilized host cell polypeptide is detected using a labeled anti-phosphotyrosine antibody. (See, King et al., 1993, Life Sciences 53:1465-1472). In yet another approach, ion flux, such as calcium ion flux, can be measured as an end point 35 for HGPRBMY2 stimulated signal transduction.

In addition, animal-based cardiovascular disorder systems may for example be used to identify compounds capable of ameliorating cardiovascular disorder-like symptoms. Such animal models may be used as test substrates for the identification of 35 drugs, pharmaceuticals, therapies and interventions which may be effective in treating such disorders. For example, animal models may be exposed to a compound, suspected of exhibiting an ability to ameliorate cardiovascular disorder symptoms, at a sufficient

concentration and for a time sufficient to elicit such an amelioration of cardiovascular disorder symptoms in the exposed animals. The response of the animals to the exposure may be monitored by assessing the reversal of disorders associated with cardiovascular disorders such as congestive heart failure. With regard to intervention, any treatments which reverse any aspect of cardiovascular disorder-like symptoms should be considered as candidates for human cardiovascular disorder therapeutic intervention. Dosages of test agents may be determined by deriving dose-response curves, as discussed in Section 10 5.7.1, below.

### **5.6. The Treatment of Immune related, Including Immune Disorders**

15 The invention features methods and compositions for modifying immune related disorders and treating immune disorders, including but not limited to immunodeficiency. Because a loss of normal HGPRBMY1 gene product function results in the development of immune related disease, an increase in HGPRBMY1 gene product activity, or activation of the HGPRBMY1 pathway (*e.g.*, downstream activation) would facilitate 20 progress towards a normal immune related state in individuals exhibiting a deficient level of HGPRBMY1 gene expression and/or HGPRBMY1 activity.

25 Alternatively, symptoms of certain immune disorders such as, for example, immunodeficiency may be ameliorated by modulating (increasing or decreasing) the level of HGPRBMY1 gene expression, and/or HGPRBMY1 gene activity, and/or modulating activity of the HGPRBMY1 pathway (*e.g.*, by targeting downstream signaling events). Different approaches are discussed below.

30 HGPRBMY1 is expressed in bone marrow, spleen and thymus tissues, thus HGPRBMY1 nucleic acids, polypeptides, and modulators thereof can be used to modulate the proliferation, development, differentiation, and/or function of immune cells, *e.g.* B-cells, dendritic cells, natural killer cells and monocytes, and/or immune function. HGPRBMY1 nucleic acids, polypeptides and modulators thereof can be utilized to modulate immune-related processes, *e.g.*, the host immune response by, for example, 35 modulating the formation of and/or binding to immune complexes, detection and defense against surface antigens and bacteria, and immune surveillance for rapid removal or pathogens.

HGPRBMY1 nucleic acids, polypeptides and modulators thereof can be utilized  
5 to modulate or treat immune disorders that include, but are not limited to, immune  
proliferative disorders (e.g., carcinoma, lymphoma, e.g., follicular lymphoma), and  
disorders associated with fighting pathogenic infections, (e.g., bacterial (e.g., chlamydia)  
infection, parasitic infection, and viral infection (e.g., HSV or HIV infection)), and  
10 pathogenic disorders (e.g., immunodeficiency disorders, such as HIV), autoimmune  
disorders, such as arthritis, multiple sclerosis, Grave's disease, or Hashimoto's disease,  
T cell disorders (e.g., AIDS) and inflammatory disorders, such as septicemia, cerebral  
malaria, inflammatory bowel disease, arthritis (e.g., rheumatoid arthritis, osteoarthritis),  
and allergic inflammatory disorders (e.g., asthma, psoriasis), apoptotic disorders (e.g.,  
15 rheumatoid arthritis, systemic lupus erythematosus, insulin-dependent diabetes mellitus),  
cytotoxic disorders, septic shock, and cachexia.

HGPRBMY1 nucleic acids, polypeptides and modulators thereof can be utilized  
to regulate immune activation to suppress rejection of a grafted organ or grafted tissue  
in a graft recipient (e.g., to prevent allograft rejection).

20 HGPRBMY1 nucleic acids, polypeptides and modulators thereof can be utilized  
to modulate immune activation. For example, antagonists to HGPRBMY1 action, such  
as peptides, antibodies or small molecules that decrease or block HGPRBMY1 activity,  
e.g., binding to extracellular matrix components, e.g., integrins, or that prevent  
25 HGPRBMY1 signaling, can be used as immune system activation blockers. In another  
example, agonists that mimic or partially mimic HGPRBMY1 activity, such as peptides,  
antibodies or small molecules, can be used to induce immune system activation.  
Antibodies may activate or inhibit the cell adhesion, proliferation and activation, and may  
help in treating infection, autoimmunity, inflammation, and cancer by affecting these  
30 cellular processes.

HGPRBMY1 nucleic acids, polypeptides and modulators thereof can also be  
utilized to modulate intercellular signaling in the immune system, e.g., modulate  
intercellular signal transduction in immune stimulation or suppression and modulate  
immune cell membrane adhesion to extra-cellular matrix components.

35 As HGPRBMY1 is expressed in bone marrow, HGPRBMY1 nucleic acids,  
polypeptides, and modulators thereof can be used to diagnose disorders associated with  
cells in the bone marrow and/or modulate the proliferation, differentiation, and/or

function of cells that appear in the bone marrow, *e.g.*, stem cells (*e.g.*, hematopoietic stem 5 cells), and blood cells, *e.g.*, erythrocytes, platelets, and leukocytes. Thus HGPRBMY1 nucleic acids, polypeptides, and modulators thereof can be used to treat bone marrow, blood, and hematopoietic associated diseases and disorders, *e.g.*, acute myeloid leukemia, hemophilia, leukemia, anemia (*e.g.*, sickle cell anemia), and thalassemia.

As HGPRBMY1 is expressed in the thymus, HGPRBMY1 nucleic acids, 10 polypeptides, and modulators thereof can be used to diagnose thymus associated disorders. HGPRBMY1 nucleic acids, polypeptides, and modulators thereof can also be used modulate the proliferation, development, differentiation, maturation and/or function of thymocytes, *e.g.*, modulate development and maturation of T-lymphocytes. 15 HGPRBMY1 nucleic acids, polypeptides and modulators thereof can be utilized to modulate immune-related processes such as the ability to modulate host immune response by, *e.g.*, modulating the formation of and/or binding to immune complexes, and modulating the positive and negative selection of thymocytes. Such HGPRBMY1 compositions and modulators thereof can be utilized, *e.g.*, to ameliorate incidence of any 20 symptoms associated with disorders that involve such immune-related processes, including, but not limited to infection and autoimmune disorders (*e.g.*, insulin-dependent mellitus, multiple sclerosis, systemic lupus, erythematosus, sjogren's syndrome, autoimmune thyroiditis, idiopathic Addison's disease, vitiligo, Grave's disease, 25 idiopathic thrombocytopenia purpura, rheumatoid arthritis, and scleroderma). HGPRBMY1 nucleic acids, polypeptides and modulators thereof can also be utilized to treat viral infections, inflammatory immune disorders and immune-related cancers including but not limited to, leukemia (*e.g.*, acute leukemia, chronic leukemia, Hodgkin's disease non-Hodgkin's lymphoma ,and multiple myeloma).

30 HGPRBMY1 has structural homology with the receptor for the serine protease, thrombin. As such HGPRBMY1 nucleic acids, polypeptides and modulators thereof can be utilized to modulate activities, processes or disorders associated with protease activity, *e.g.*, serine protease activity. For example, HGPRBMY1 nucleic acids, polypeptides or modulators thereof can be used to modulate serine protease activities, such as those 35 activities associated with such serine proteases (or, where appropriate, human homologues thereof), *e.g.*, adipsin (complement factor D), acrosin, thrombin, plasminogen, protein C, cathepsin G, chymotrypsin, complement components and

signaling, cytotoxic cell proteases, duodenase I, elastases 1, 2, 3A, 3B and medullasin, 5 enterokinase, hepatocyte growth factor activator, hepsin, kallikreins, gamma-renin, prostate specific antigen, mast cell proteases, myeloblastin, Alzheimer's plaque-related proteases, tryptases, ancrad, batroxobin, cerastobin, flavoxobin, apolipoprotein, blood fluke cercarial protease, *Drosophila* trypsin like protease (e.g., alpha, easter, and snake locus), *Drosophila* protease stubble, or major mite fecal antigen.

10 HGPRBMY1 nucleic acids, polypeptides and modulators thereof can be used to modulate processes and/or diseases involved with serine protease response activity. For example, such processes and/or diseases can include, but are not limited to cellular activation, cellular proliferation, motility and differentiation, the alternative complement 15 pathway, e.g., disturbances of the complement regulation system, such as complement regulator deficiencies, which include, for example, hereditary angioedema (an allergic disorder) and proxysmal nocturnal hemoglobinuria (the presence of hemoglobin in the urine), modulate body weight or body weight disorders, e.g., obesity or cachexia, systemic energy balance and diabetes.

20 In addition, assays can be developed to measure the biological activity of polypeptides or peptides of the invention. In particular, HGPRBMY1 or modulators thereof, biological activities include, e.g., (1) the ability to modulate development, differentiation, proliferation and/or activity of immune cells (e.g., leukocytes and 25 macrophages), endothelial cells and smooth muscle cells; (2) the ability to modulate the host immune response; (3) the ability to modulate intracellular signaling cascades (e.g., signal transduction cascades); (4) the ability to modulate the development of organs, tissues and/or cells of the embryo and/or fetus; (5) the ability to modulate cell-cell interactions and/or cell-extracellular matrix interactions; (6) the ability to modulate 30 atherosclerosis, e.g., the initiation and progression of atherosclerosis; (7) the ability to modulate atherogenesis; (8) the ability to modulate inflammatory functions e.g., by modulating leukocyte adhesion to extracellular matrix and/or endothelial cells; (9) the ability to bind and phagocytose cells, e.g., aged and apoptotic cells; (10) the ability to 35 remove debris, e.g., apoptotic cells, from blood vessel walls; (11) the ability to modulate, e.g., inhibit, the expression of molecules, e.g., adhesion molecules (e.g., leukocyte adhesion molecules) and growth factors (e.g., smooth-muscle growth factors); (12) the ability to alter, e.g., increase, expression in response to stimuli, e.g., TNF, shear stress,

and pathophysiological stimuli relevant to disorders (e.g., atherosclerosis and 5 inflammation); and (13) the ability to form, e.g., stabilize, promote, facilitate, inhibit, or disrupt, cell to cell and cell to blood product interaction, e.g., between leukocytes and platelets or leukocytes and vascular endothelial cells.

10 **5.6b. The Treatment of Cardiovascular, Including Cardiovascular Disorders**

The invention encompasses methods and compositions for modifying cardiovascular and treating cardiovascular disorders, including but not limited to congestive heart failure. Because a loss of normal HGPRBMY2 gene product function results in the development of cardiovascular disease, an increase in HGPRBMY2 gene 15 product activity, or activation of the HGPRBMY2 pathway (e.g., downstream activation) would facilitate progress towards a normal cardiovascular state in individuals exhibiting a deficient level of HGPRBMY2 gene expression and/or HGPRBMY2 activity.

Alternatively, symptoms of certain cardiovascular disorders such as, for example, 20 congestive heart failure may be ameliorated by modulating (increasing or decreasing) the level of HGPRBMY2 gene expression, and/or HGPRBMY2 gene activity, and/or modulating activity of the HGPRBMY2 pathway (e.g., by targeting downstream signalling events). Different approaches are discussed below.

25 **5.6c. The Treatment of Neurological Disorders and Diseases**

Nervous system diseases, disorders, and/or conditions, which can be treated, prevented, and/or diagnosed with the compositions of the invention (e.g., HGPRBMY2 polypeptides, polynucleotides, and/or agonists or antagonists), include, but are not limited to, nervous system injuries, and diseases, disorders, and/or conditions which result in 30 either a disconnection of axons, a diminution or degeneration of neurons, or demyelination. Nervous system lesions which may be treated, prevented, and/or diagnosed in a patient (including human and non-human mammalian patients) according to the invention, include but are not limited to, the following lesions of either the central (including spinal cord, brain) or peripheral nervous systems: (1) ischemic lesions, in 35 which a lack of oxygen in a portion of the nervous system results in neuronal injury or death, including cerebral infarction or ischemia, or spinal cord infarction or ischemia; (2) traumatic lesions, including lesions caused by physical injury or associated with surgery,

for example, lesions which sever a portion of the nervous system, or compression  
5 injuries; (3) malignant lesions, in which a portion of the nervous system is destroyed or  
injured by malignant tissue which is either a nervous system associated malignancy or a  
malignancy derived from non-nervous system tissue; (4) infectious lesions, in which a  
portion of the nervous system is destroyed or injured as a result of infection, for example,  
10 by an abscess or associated with infection by human immunodeficiency virus, herpes  
zoster, or herpes simplex virus or with Lyme disease, tuberculosis, syphilis; (5)  
degenerative lesions, in which a portion of the nervous system is destroyed or injured as  
a result of a degenerative process including but not limited to degeneration associated  
15 with Parkinson's disease, Alzheimer's disease, Huntington's chorea, or amyotrophic  
lateral sclerosis (ALS); (6) lesions associated with nutritional diseases, disorders, and/or  
conditions, in which a portion of the nervous system is destroyed or injured by a  
nutritional disorder or disorder of metabolism including but not limited to, vitamin B12  
deficiency, folic acid deficiency, Wernicke disease, tobacco-alcohol amblyopia,  
20 Marchiafava-Bignami disease (primary degeneration of the corpus callosum), and  
alcoholic cerebellar degeneration; (7) neurological lesions associated with systemic  
diseases including, but not limited to, diabetes (diabetic neuropathy, Bell's palsy),  
systemic lupus erythematosus, carcinoma, or sarcoidosis; (8) lesions caused by toxic  
substances including alcohol, lead, or particular neurotoxins; and (9) demyelinated  
25 lesions in which a portion of the nervous system is destroyed or injured by a  
demyelinating disease including, but not limited to, multiple sclerosis, human  
immunodeficiency virus-associated myelopathy, transverse myelopathy or various  
etiologies, progressive multifocal leukoencephalopathy, and central pontine myelinolysis.

In a preferred embodiment, the HGPRBMY2 polypeptides, polynucleotides, or  
30 agonists or antagonists of the invention are used to protect neural cells from the damaging  
effects of cerebral hypoxia. According to this embodiment, the compositions of the  
invention are used to treat, prevent, and/or diagnose neural cell injury associated with  
cerebral hypoxia. In one aspect of this embodiment, the polypeptides, polynucleotides,  
35 or agonists or antagonists of the invention are used to treat, prevent, and/or diagnose  
neural cell injury associated with cerebral ischemia. In another aspect of this  
embodiment, the polypeptides, polynucleotides, or agonists or antagonists of the  
invention are used to treat, prevent, and/or diagnose neural cell injury associated with

cerebral infarction. In another aspect of this embodiment, the polypeptides, 5 polynucleotides, or agonists or antagonists of the invention are used to treat, prevent, and/or diagnose or prevent neural cell injury associated with a stroke. In a further aspect of this embodiment, the polypeptides, polynucleotides, or agonists or antagonists of the invention are used to treat, prevent, and/or diagnose neural cell injury associated with a heart attack.

10

The HGPRBMY2 compositions of the invention which are useful for treating or preventing a nervous system disorder may be selected by testing for biological activity in promoting the survival or differentiation of neurons. For example, and not by way of limitation, compositions of the invention which elicit any of the following effects may 15 be useful according to the invention: (1) increased survival time of neurons in culture; (2) increased sprouting of neurons in culture or in vivo; (3) increased production of a neuron-associated molecule in culture or in vivo, e.g., choline acetyltransferase or acetylcholinesterase with respect to motor neurons; or (4) decreased symptoms of neuron dysfunction in vivo. Such effects may be measured by any method known in the art. In 20 preferred, non-limiting embodiments, increased survival of neurons may routinely be measured using a method set forth herein or otherwise known in the art, such as, for example, the method set forth in Arakawa et al. (J. Neurosci. 10:3507-3515 (1990)); increased sprouting of neurons may be detected by methods known in the art, such as, for 25 example, the methods set forth in Pestronk et al. (Exp. Neurol. 70:65-82 (1980)) or Brown et al. (Ann. Rev. Neurosci. 4:17-42 (1981)); increased production of neuron-associated molecules may be measured by bioassay, enzymatic assay, antibody binding, Northern blot assay, etc., using techniques known in the art and depending on the molecule to be measured; and motor neuron dysfunction may be measured by assessing 30 the physical manifestation of motor neuron disorder, e.g., weakness, motor neuron conduction velocity, or functional disability.

In specific embodiments, motor neuron diseases, disorders, and/or conditions that 35 may be treated, prevented, and/or diagnosed according to the invention include, but are not limited to, diseases, disorders, and/or conditions such as infarction, infection, exposure to toxin, trauma, surgical damage, degenerative disease or malignancy that may affect motor neurons as well as other components of the nervous system, as well as diseases, disorders, and/or conditions that selectively affect neurons such as amyotrophic

lateral sclerosis, and including, but not limited to, progressive spinal muscular atrophy,  
5 progressive bulbar palsy, primary lateral sclerosis, infantile and juvenile muscular atrophy, progressive bulbar paralysis of childhood (Fazio-Londe syndrome), poliomyelitis and the post polio syndrome, and Hereditary Motorsensory Neuropathy (Charcot-Marie-Tooth Disease).

10

#### 5.6.1. Modulation of HGPRBMY1 Expression or Activity

Any method which neutralizes an agonist or antagonist or modulates expression of the HGPRBMY1 gene (e.g., by either activating or decreasing transcription or translation) can be used to prevent HGPRBMY1 immune disorders.

15

For example, the administration of soluble peptides, polypeptides, fusion polypeptides, or antibodies (including anti-idiotypic antibodies) that bind to a circulating agonist or antagonist, the natural ligand for the HGPRBMY1, can be used to prevent or treat immune disorders. To this end, peptides corresponding to the ECD of HGPRBMY1, soluble deletion mutants of HGPRBMY1 (e.g.,  $\Delta$ TM-HGPRBMY1 mutants), or either 20 of these HGPRBMY1 domains or mutants fused to another polypeptide (e.g., an IgFc polypeptide) can be utilized. Alternatively, anti-idiotypic antibodies or Fab fragments of antiidiotypic antibodies that mimic the HGPRBMY1 ECD and neutralize agonists or antagonists can be used (see Section 5.3, supra). Such HGPRBMY1 polypeptides, 25 peptides, fusion polypeptides, anti-idiotypic antibodies or Fabs are administered to a subject in amounts sufficient to neutralize agonist or antagonist and to prevent or treat immune disorders.

25

Fusion of the HGPRBMY1, the HGPRBMY1 ECD or the  $\Delta$ TMHGPRBMY1 to an IgFc polypeptide should not only increase the stability of the preparation, but will 30 increase the half-life and activity of the HGPRBMY1-Ig fusion polypeptide *in vivo*. The Fc region of the Ig portion of the fusion polypeptide may be further modified to reduce immunoglobulin effector function. In an alternative embodiment for neutralizing circulating agonist or antagonist, cells that are genetically engineered to express such 35 soluble or secreted forms of HGPRBMY1 may be administered to a patient, whereupon they will serve as "bioreactors" *in vivo* to provide a continuous supply of the agonist or antagonist neutralizing polypeptide. Such cells may be obtained from the patient or an MHC compatible donor and can include, but are not limited to fibroblasts, blood cells

(e.g., lymphocytes), adipocytes, muscle cells, endothelial cells etc. The cells are 5 genetically engineered *in vitro* using recombinant DNA techniques to introduce the coding sequence for the HGPRBMY1 ECD,  $\Delta$ TMHGPRBMY1, or for HGPRBMY1-Ig fusion polypeptide (e.g., HGPRBMY1-, ECD- or  $\Delta$ TMHGPRBMY1-IgFc fusion polypeptides) into the cells, etc. by transduction (using viral vectors, and preferably 10 vectors that integrate the transgene into the cell genome) or transfection procedures, including but not limited to the use of plasmids, cosmids, YACs, electroporation, liposomes, etc. The HGPRBMY1 coding sequence can be placed under the control of a strong constitutive or inducible promoter or promoter/enhancer to achieve expression and 15 secretion of the HGPRBMY1 peptide or fusion polypeptide. The engineered cells which express and secrete the desired HGPRBMY1 product can be introduced into the patient systemically, e.g., in the circulation, intraperitoneally, at the heart. Alternatively, the cells can be incorporated into a matrix and implanted in the body, e.g., genetically engineered 20 fibroblasts can be implanted as part of a skin graft; genetically engineered endothelial cells can be implanted as part of a vascular graft (See, for example, Anderson et al. U.S. Pat. No. 5,399,349; and Mulligan & Wilson, U.S. Pat. No. 5,460,959 each of which is incorporated by reference herein in its entirety).

When the cells to be administered are non-autologous cells, they can be administered using well known techniques which prevent the development of a host 25 immune response against the introduced cells. For example, the cells may be introduced in an encapsulated form which, while allowing for an exchange of components with the immediate extracellular environment, does not allow the introduced cells to be recognized by the host immune system.

In an alternate embodiment, immune disorder therapy can be designed to reduce 30 the level of endogenous HGPRBMY1 gene expression, e.g., using antisense or ribozyme approaches to inhibit or prevent translation of HGPRBMY1 mRNA transcripts; triple helix approaches to inhibit transcription of the HGPRBMY1 gene; or targeted 35 homologous recombination to inactivate or "knock out" the HGPRBMY1 gene or its endogenous promoter. Alternatively, the antisense, ribozyme or DNA constructs described herein could be administered directly to the site containing the target cells; e.g., the bone marrow or spleen.

Antisense approaches involve the design of oligonucleotides (either DNA or RNA) that are complementary to HGPRBMY1 mRNA. The antisense oligonucleotides will bind to the complementary HGPRBMY1 mRNA transcripts and prevent translation. Absolute complementarity, although preferred, is not required. A sequence "complementary" to a portion of an RNA, as referred to herein, means a sequence having sufficient complementarity to be able to hybridize with the RNA, forming a stable duplex; in the case of double-stranded antisense nucleic acids, a single strand of the duplex DNA may thus be tested, or triplex formation may be assayed. The ability to hybridize will depend on both the degree of complementarity and the length of the antisense nucleic acid. Generally, the longer the hybridizing nucleic acid, the more base mismatches with an RNA it may contain and still form a stable duplex (or triplex, as the case may be). One skilled in the art can ascertain a tolerable degree of mismatch by use of standard procedures to determine the melting point of the hybridized complex.

Oligonucleotides that are complementary to the 5' end of the message, *e.g.*, the 5' untranslated sequence up to and including the AUG initiation codon, should work most efficiently at inhibiting translation. However, sequences complementary to the 3' untranslated sequences of mRNAs have recently shown to be effective at inhibiting translation of mRNAs as well. See generally, Wagner, R., 1994, *Nature* 372:333-335. Thus, oligonucleotides complementary to either the 5'- or 3'- non-translated, non-coding regions of the HGPRBMY1 shown in SEQ ID NO:1, could be used in an antisense approach to inhibit translation of endogenous HGPRBMY1 mRNA. Oligonucleotides complementary to the 5' untranslated region of the mRNA should include the complement of the AUG start codon. Antisense oligonucleotides complementary to mRNA coding regions are less efficient inhibitors of translation but could be used in accordance with the invention. Whether designed to hybridize to the 5'-, 3'- or coding region of HGPRBMY1 mRNA, antisense nucleic acids should be at least six nucleotides in length, and are preferably oligonucleotides ranging from 6 to about 50 nucleotides in length. In specific aspects the oligonucleotide is at least nucleotides, at least 17 nucleotides, at least 25 nucleotides or at least 50 nucleotides.

35        Regardless of the choice of target sequence, it is preferred that *in vitro* studies are first performed to quantitate the ability of the antisense oligonucleotide to inhibit gene expression. It is preferred that these studies utilize controls that distinguish between

antisense gene inhibition and nonspecific biological effects of oligonucleotides. It is also preferred that these studies compare levels of the target RNA or polypeptide with that of an internal control RNA or polypeptide. Additionally, it is envisioned that results obtained using the antisense oligonucleotide are compared with those obtained using a control oligonucleotide. It is preferred that the control oligonucleotide is of approximately the same length as the test oligonucleotide and that the nucleotide sequence of the oligonucleotide differs from the antisense sequence no more than is necessary to prevent specific hybridization to the target sequence.

The oligonucleotides can be DNA or RNA or chimeric mixtures or derivatives or modified versions thereof, single-stranded or double-stranded. The oligonucleotide can be modified at the base moiety, sugar moiety, or phosphate backbone, for example, to improve stability of the molecule, hybridization, etc. The oligonucleotide may include other appended groups such as peptides (*e.g.*, for targeting host cell receptors *in vivo*), or agents facilitating transport across the cell membrane (see, *e.g.*, Letsinger et al., 1989, Proc. Natl. Acad. Sci. U.S.A. 86:6553-6556; Lemaitre et al., 1987, Proc. Natl. Acad. Sci. 84:648-652; PCT Publication No. WO88/09810, published Dec. 15, 1988), hybridization-triggered cleavage agents (See, *e.g.*, Krol et al., 1988, BioTechniques 6:958-976) or intercalating agents (See, *e.g.*, Zon, 1988, Pharm. Res. 5:539-549). To this end, the oligonucleotide may be conjugated to another molecule, *e.g.*, a peptide, hybridization triggered cross-linking agent, transport agent, hybridization-triggered cleavage agent, etc.

The antisense oligonucleotide may comprise at least one modified base moiety which is selected from the group including but not limited to 5-fluorouracil, 5-bromouracil, 5-chlorouracil, 5-iodouracil, hypoxanthine, xanthine, 4-acetylcytosine, 5-(carboxyhydroxymethyl) uracil, 5-carboxymethylaminomethyl-2-thiouridine, 5-carboxymethylaminomethyluracil, dihydrouracil,  $\beta$ -D-galactosylqueosine, inosine, N6-isopentenyladenine, 1-methylguanine, 1-methylinosine, 2,2-dimethylguanine, 2-methyladenine, 2-methylguanine, 3-methylcytosine, 5-methylcytosine, N6-adenine, 7-methylguanine, 5-methylaminomethyluracil, 5-methoxyaminomethyl-2-thiouracil,  $\beta$ -D-mannosylqueosine, 5'-methoxycarboxymethyluracil, 5-methoxyuracil, 2-methylthio-N6-isopentenyladenine, uracil-5-oxyacetic acid (v), wybutoxosine, pseudouracil, queosine, 2-thiacytosine, 5-methyl-2-thiouracil, 2-thiouracil, 4-thiouracil,

5-methyluracil, uracil 5-oxyacetic acid methylester, uracil-5-oxyacetic acid (v), 5-methyl-2-thiouracil, 3-(3-amino-3-N-2-carboxypropyl) uracil, (acp3)w, and 2,6-diaminopurine. The antisense oligonucleotide may also comprise at least one modified sugar moiety selected from the group including but not limited to arabinose, 2-fluoroarabinose, xylulose, and hexose.

In yet another embodiment, the antisense oligonucleotide comprises at least one modified phosphate backbone selected from the group consisting of a phosphorothioate, a phosphorodithioate, a phosphoramidothioate, a phosphoramidate, a phosphordiamidate, a methylphosphonate, an alkyl phosphotriester, and a formacetal or analog thereof.

In yet another embodiment, the antisense oligonucleotide is an  $\alpha$ -anomeric oligonucleotide. An  $\alpha$ -anomeric oligonucleotide forms specific double-stranded hybrids with complementary RNA in which, contrary to the usual  $\beta$ -units, the strands run parallel to each other (Gautier et al., 1987, Nucl. Acids Res. 15:6625-6641). The oligonucleotide is a 2'-0-methylribonucleotide (Inoue et al., 1987, Nucl. Acids Res. 15:6131-6148), or a chimeric RNA-DNA analogue (Inoue et al., 1987, FEBS Lett. 215:327-330).

Oligonucleotides of the invention may be synthesized by standard methods known in the art, e.g. by use of an automated DNA synthesizer (such as are commercially available from Biosearch, Applied Biosystems, etc.). As examples, phosphorothioate oligonucleotides may be synthesized by the method of Stein et al. (1988, Nucl. Acids Res. 16:3209), methylphosphonate oligonucleotides can be prepared by use of controlled pore glass polymer supports (Sarin et al., 1988, Proc. Natl. Acad. Sci. U.S.A. 85:7448-7451), etc. While antisense nucleic acids complementary to the HGPRBMY1 coding region sequence could be used, those complementary to the transcribed untranslated region are most preferred. For example, antisense oligonucleotides having the following sequences can be utilized in accordance with the invention:

- a) 5'-CATCCGCCTTATTACAT-3' (SEQ ID NO:28) which is complementary to nucleotides -14 to +3 as shown in SEQ ID NO:1;
- b) 5'-CATCCGCCTTATTACATCTTTT-3' (SEQ ID NO:29) which is complementary to nucleotides -20 to +3 in SEQ ID NO:1.

The antisense molecules should be delivered to cells which express the HGPRBMY1 *in vivo*, e.g., the bone marrow or spleen. A number of methods have been developed for delivering antisense DNA or RNA to cells; e.g., antisense molecules can

be injected directly into the tissue site, or modified antisense molecules, designed to target the desired cells (e.g., antisense linked to peptides or antibodies that specifically bind receptors or antigens expressed on the target cell surface) can be administered systemically.

However, it is often difficult to achieve intracellular concentrations of the antisense sufficient to suppress translation of endogenous mRNAs. Therefore a preferred approach utilizes a recombinant DNA construct in which the antisense oligonucleotide is placed under the control of a strong pol III or pol II promoter. The use of such a construct to transfect target cells in the patient will result in the transcription of sufficient amounts of single stranded RNAs that will form complementary base pairs with the endogenous HGPRBMY1 transcripts and thereby prevent translation of the HGPRBMY1 mRNA. For example, a vector can be introduced *in vivo* such that it is taken up by a cell and directs the transcription of an antisense RNA. Such a vector can remain episomal or become chromosomally integrated, as long as it can be transcribed to produce the desired antisense RNA. Such vectors can be constructed by recombinant DNA technology methods standard in the art. Vectors can be plasmid, viral, or others known in the art, used for replication and expression in mammalian cells. Expression of the sequence encoding the antisense RNA can be by any promoter known in the art to act in mammalian, preferably human cells. Such promoters can be inducible or constitutive. Such promoters include but are not limited to: the SV40 early promoter region (Benoist and Chambon, 1981, *Nature* 290:304-310), the promoter contained in the 3' long terminal repeat of Rous sarcoma virus (Yamamoto et al., 1980, *Cell* 22:787-797), the herpes thymidine kinase promoter (Wagner et al., 1981, *Proc. Natl. Acad. Sci. U.S.A.* 78:1441-1445), the regulatory sequences of the metallothionein gene (Brinster et al., 1982, *Nature* 296:39-42), etc. Any type of plasmid, cosmid, YAC or viral vector can be used to prepare the recombinant DNA construct which can be introduced directly into the tissue site; e.g., the bone marrow or spleen. Alternatively, viral vectors can be used which selectively infect the desired tissue; (e.g., for bone marrow or spleen, herpesvirus vectors may be used or alternatively, in dividing bone marrow cells retroviruses may be used), in which case administration may be accomplished by another route (e.g., systemically).

Ribozyme molecules-designed to catalytically cleave HGPRBMY1 mRNA transcripts can also be used to prevent translation of HGPRBMY1mRNA and expression

of HGPRBMY1. (See, *e.g.*, PCT International Publication WO90/11364, published Oct. 4, 1990; Sarver et al., 1990, *Science* 247:1222-1225). While ribozymes that cleave mRNA at site specific recognition sequences can be used to destroy HGPRBMY1 mRNAs, the use of hammerhead ribozymes is preferred. Hammerhead ribozymes cleave mRNAs at locations dictated by flanking regions that form complementary base pairs with the target mRNA. The sole requirement is that the target mRNA have the following sequence of two bases: 5'-UG-3'. The construction and production of hammerhead ribozymes is well known in the art and is described more fully in Haseloff and Gerlach, 1988, *Nature*, 334:585-591. There are hundreds of potential hammerhead ribozyme cleavage sites within the nucleic acid sequence of human HGPRBMY1 cDNA. Preferably the ribozyme is engineered so that the cleavage recognition site is located near the 5' end of the HGPRBMY1 mRNA; *i.e.*, to increase efficiency and minimize the intracellular accumulation of non-functional mRNA transcripts. For example, hammerhead ribozymes can be utilized in accordance with the invention.

The ribozymes of the present invention also include RNA endoribonucleases (hereinafter "Cech-type ribozymes") such as the one which occurs naturally in *Tetrahymena Thermophila* (known as the IVS, or L-19 IVS RNA) and which has been extensively described by Thomas Cech and collaborators (Zaug, et al., 1984, *Science*, 224:574-578; Zaug and Cech, 1986, *Science*, 231:470-475; Zaug, et al., 1986, *Nature*, 324:429-433; published International patent-application No. WO 88/04300 by University Patents Inc.; Been and Cech, 1986, *Cell*, 47:207-216). The Cech-type ribozymes have an eight base pair active site which hybridizes to a target RNA sequence whereafter cleavage of the target RNA takes place. The invention features those Cech-type ribozymes which target eight base-pair active site sequences that are present in HGPRBMY1.

As in the antisense approach, the ribozymes can be composed of modified oligonucleotides (*e.g.* for improved stability, targeting, etc.) and should be delivered to cells which express the HGPRBMY1 *in vivo*, *e.g.*, bone marrow or spleen. A preferred method of delivery involves using a DNA construct "encoding" the ribozyme under the control of a strong constitutive pol III or pol II promoter, so that transfected cells will produce sufficient quantities of the ribozyme to destroy endogenous HGPRBMY1 messages and inhibit translation. Because ribozymes unlike antisense molecules, are catalytic, a lower intracellular concentration is required for efficiency.

Endogenous HGPRBMY1 gene expression can also be reduced by inactivating the HGPRBMY1 gene or its promoter using targeted homologous recombination (*e.g.*, see Smithies et al., 1985, *Nature* 317:230-234; Thomas & Capecchi, 1987, *Cell* 51:503-512; Thompson et al., 1989 *Cell* 5:313-321; each of which is incorporated by reference herein in its entirety). For example, a mutant, non-functional HGPRBMY1, or unrelated sequences, which are flanked by DNA homologous to the endogenous HGPRBMY1 gene locus can be used with or without a selectable marker and/or a negative selectable marker, to transfet cells that express HGPRBMY1 *in vivo*. Insertion of the DNA construct, via targeted homologous recombination, results in inactivation of the HGPRBMY1 gene. Such approaches are particularly suited in the agricultural field where modifications to ES (embryonic stem) cells can be used to generate animal offspring with an inactive HGPRBMY1 (*e.g.*, see Thomas & Capecchi 1987 and Thompson 1989, *supra*). However this approach can be adapted for use in humans provided the recombinant DNA constructs are directly administered or targeted to the required site *in vivo* using appropriate viral vectors, *e.g.*, herpes virus vectors for delivery to tissue; *e.g.*, bone marrow or spleen.

Alternatively, endogenous HGPRBMY1 gene expression can be reduced by targeting deoxyribonucleotide sequences complementary to the regulatory region of the HGPRBMY1 gene (*i.e.*, the HGPRBMY1 promoter and/or enhancers) to form triple helical structures that prevent transcription of the HGPRBMY1 gene in target cells in the body. (See generally, Helene, C. 1991, *Anticancer Drug Des.*, 6(6):569-84; Helene, C., et al., 1992, *Ann. N.Y. Accad. Sci.*, 660:27-36; and Maher, L. J., 1992, *Bioassays* 14(12):807-15).

In yet another embodiment of the invention, the activity of HGPRBMY1 can be reduced using a “dominant negative” approach to prevent or treat immune disorders. To this end, constructs which encode defective HGPRBMY1 can be used in gene therapy approaches to diminish the activity of the HGPRBMY1 in appropriate target cells. For example, nucleic acid sequences that direct host cell expression of HGPRBMY1 in which the CD is deleted or mutated can be introduced into cells in the bone marrow or spleen (either by *in vivo* or *ex vivo* gene therapy methods described above). Alternatively, targeted homologous recombination can be utilized to introduce such deletions or mutations into the subject’s endogenous HGPRBMY1 gene in the bone marrow or

spleen. The engineered cells will express non-functional receptors (*i.e.*, an anchored receptor that is capable of binding its natural ligand, but incapable of signal transduction). Such engineered cells present in the bone marrow or spleen should demonstrate a diminished response to the endogenous agonist or antagonist ligand, resulting in immune disorders.

With respect to an increase in the level of normal HGPRBMY1 gene expression and/or HGPRBMY1 gene product activity, HGPRBMY1 nucleic acid sequences can be utilized for the treatment of immune disorders, including immunodeficiency. Where the cause of immunodeficiency is a defective HGPRBMY1, treatment can be administered, for example, in the form of gene replacement therapy.

In another embodiment, the expression characteristics of an endogenous gene (*e.g.*, HGPRBMY1 genes) within a cell, cell line or microorganism may be modified by inserting a DNA regulatory element heterologous to the endogenous gene of interest into the genome of a cell, stable cell line or cloned microorganism such that the inserted regulatory element is operatively linked with the endogenous gene (*e.g.*, HGPRBMY1 genes) and controls, modulates or activates. For example, endogenous HGPRBMY1 genes which are normally "transcriptionally silent", *i.e.*, a HGPRBMY1 genes which is normally not expressed, or are expressed only at very low levels in a cell line or microorganism, may be activated by inserting a regulatory element which is capable of promoting the expression of a normally expressed gene product in that cell line or microorganism. Alternatively, transcriptionally silent, endogenous HGPRBMY1 genes may be activated by insertion of a promiscuous regulatory element that works across cell types.

A heterologous regulatory element may be inserted into a stable cell line or cloned microorganism, such that it is operatively linked with and activates expression of endogenous HGPRBMY1 genes, using techniques, such as targeted homologous recombination, which are well known to those of skill in the art, and described *e.g.*, in Chappel, U.S. Patent No. 5,272,071; PCT publication No. WO 91/06667, published May 16, 1991; Skoulchi U.S. Patent No. 5,981,214; Treco *et al* U.S. Patent No. 5,968,502 and PCT publication No. WO 94/12650, published June 9, 1994. Alternatively, non-targeted *e.g.*, non-homologous recombination techniques which are well-known to those of skill

in the art and described, *e.g.*, in PCT publication No. WO 99/15650, published April 1, 1999, may be used.

Specifically, one or more copies of a normal HGPRBMY1 gene or a portion of the HGPRBMY1 gene that directs the production of an HGPRBMY1 gene product exhibiting normal function, may be inserted into the appropriate cells within a patient or animal subject, using vectors which include, but are not limited to adenovirus, adeno-associated virus, retrovirus and herpes virus vectors, in addition to other particles that introduce DNA into cells, such as liposomes.

Because the HGPRBMY1 gene is expressed in the bone marrow, spleen and thymus, such gene replacement therapy techniques should be capable of delivering HGPRBMY1 gene sequences to these cell types within patients. Thus, the techniques for delivery of the HGPRBMY1 gene sequences should be designed to readily involve direct administration of such HGPRBMY1 gene sequences to the site of the cells in which the HGPRBMY1 gene sequences are to be expressed. Alternatively, targeted homologous recombination can be utilized to correct the defective endogenous HGPRBMY1 gene in the appropriate tissue; *e.g.*, bone marrow or spleen cells (particularly B-cells). In animals, targeted homologous recombination can be used to correct the defect in ES cells in order to generate offspring with a corrected trait.

Additional methods which may be utilized to increase the overall level of HGPRBMY1 gene expression and/or HGPRBMY1 activity include the introduction of appropriate HGPRBMY1-expressing cells, preferably autologous cells, into a patient at positions and in numbers which are sufficient to ameliorate the symptoms of immune disorders, including immunodeficiency. Such cells may be either recombinant or non-recombinant. Among the cells which can be administered to increase the overall level of HGPRBMY1 gene expression in a patient are normal cells, preferably bone marrow or spleen cells, cells which express the HGPRBMY1 gene. The cells can be administered at the anatomical site in the body, or as part of a tissue graft located at a different site in the body. Such cell-based gene therapy techniques are well known to those skilled in the art, see, *e.g.*, Anderson, et al., U.S. Pat. No. 5,399,349; Mulligan & Wilson, U.S. Pat. No. 5,460,959.

Finally, compounds, identified in the assays described above, that stimulate or enhance the signal transduced by activated HGPRBMY1, *e.g.*, by activating downstream

signaling polypeptides in the HGPRBMY1 cascade and thereby by-passing the defective HGPRBMY1, can be used to ameliorate immune related disease. The formulation and mode of administration will depend upon the physico-chemical properties of the compound.

#### **5.6.1b. Modulation of HGPRBMY2 Expression or HGPRBMY2 Activity to Prevent Heart Failure**

Any method which neutralizes an agonist or antagonist or modulates expression of the HGPRBMY2 gene (either transcription or translation) can be used to prevent heart failure or heart disease. Such approaches can be used to treat any cardiovascular disorder.

For example, the administration of soluble peptides, polypeptides, fusion polypeptides, or antibodies that bind to the natural ligand for the HGPRBMY2, can be used to prevent or treat heart disease. To this end, peptides corresponding to the ECD of HGPRBMY2, soluble deletion mutants of HGPRBMY2 (e.g.,  $\Delta$ TM-HGPRBMY2 mutants), or either of these HGPRBMY2 domains or mutants fused to another polypeptide (e.g., an IgFc polypeptide) can be utilized. Alternatively, anti-idiotypic antibodies or fragments thereof that mimic the HGPRBMY2 ECD and neutralize agonists or antagonists can be used (see Section 5.3, supra). Such HGPRBMY2 polypeptides, peptides, fusion polypeptides, and/or antibodies are administered to a subject in amounts sufficient to bind the ligand and to prevent or treat heart disease.

Fusion of the HGPRBMY2, the HGPRBMY2-ECD to an IgFc polypeptide should not only increase the stability of the preparation, but will increase the half-life and activity of the HGPRBMY2-Ig fusion polypeptide *in vivo*. The Fc region of the Ig portion of the fusion polypeptide may be further modified to reduce immunoglobulin effector function. In an alternative embodiment for neutralizing circulating agonist or antagonist, cells that are genetically engineered to express such soluble or secreted forms of HGPRBMY2 may be administered to a patient to provide a continuous supply of the agonist or antagonist neutralizing polypeptide. Such cells may be obtained from the patient or an MHC compatible donor and can include, but are not limited to fibroblasts, blood cells (e.g., lymphocytes), adipocytes, muscle cells, endothelial cells etc. The cells are genetically engineered *in vitro* using recombinant DNA techniques to introduce the coding sequence for the HGPRBMY2 ECD,  $\Delta$ TMHGPRBMY2, or for HGPRBMY2-Ig

fusion polypeptide (*e.g.*, HGPRBMY2-, ECD- or  $\Delta$ TMHGPRBMY2-IgFc fusion polypeptides) into the cells, etc. by transduction (using viral vectors, and preferably vectors that integrate the transgene into the cell genome) or transfection procedures, including but not limited to the use of plasmids, cosmids, YACs, electroporation, liposomes, etc.

The HGPRBMY2 coding sequence can be placed under the control of a strong constitutive or inducible promoter or promoter/enhancer to achieve expression and secretion of the HGPRBMY2 peptide or fusion polypeptide. The engineered cells which express and secrete the desired HGPRBMY2 product can be introduced into the patient systemically, *e.g.*, in the circulation, intraperitoneally, at the heart. Alternatively, the cells can be incorporated into a matrix and implanted in the body, *e.g.*, genetically engineered fibroblasts can be implanted as part of a skin graft; genetically engineered endothelial cells can be implanted as part of a vascular graft. (See, for example, Anderson et al. 5,399,349; and Mulligan & Wilson, 5,460,959 each of which is incorporated by reference herein in its entirety).

When the cells to be administered are non-autologous cells, they can be administered using well known techniques which prevent the development of a host immune response against the introduced cells. For example, the cells may be introduced in an encapsulated form which, while allowing for an exchange of components with the immediate extracellular environment, does not allow the introduced cells to be recognized by the host immune system.

In an alternate embodiment, heart failure therapy can be designed to reduce the level of endogenous HGPRBMY2 gene expression, *e.g.*, using antisense or ribozyme approaches to inhibit or prevent translation of HGPRBMY2 mRNA transcripts; triple helix approaches to inhibit transcription of the HGPRBMY2 gene; or targeted homologous recombination to inactivate or "knock out" the HGPRBMY2 gene or its endogenous promoter. Alternatively, the antisense, ribozyme or DNA constructs described herein could be administered directly to the site containing the target cells; *e.g.*, the heart.

Antisense approaches involve the design of oligonucleotides (either DNA or RNA) that are complementary to HGPRBMY2 mRNA. The antisense oligonucleotides will bind to the complementary HGPRBMY2 mRNA transcripts and prevent translation.

Absolute complementarity, although preferred, is not required. A sequence "complementary" to a portion of an RNA, as referred to herein, means a sequence having sufficient complementarity to be able to hybridize with the RNA, forming a stable duplex; in the case of double-stranded antisense nucleic acids, a single strand of the duplex DNA may thus be tested, or triplex formation may be assayed. The ability to hybridize will depend on both the degree of complementarity and the length of the antisense nucleic acid. Generally, the longer the hybridizing nucleic acid, the more base mismatches with an RNA it may contain and still form a stable duplex (or triplex, as the case may be). One skilled in the art can ascertain a tolerable degree of mismatch by use of standard procedures to determine the melting point of the hybridized complex.

Oligonucleotides that are complementary to the 5' end of the message, *e.g.*, the 5' untranslated sequence up to and including the AUG initiation codon, should work most efficiently at inhibiting translation. However, sequences complementary to the 3' untranslated sequences of mRNAs have recently shown to be effective at inhibiting translation of mRNAs as well. See generally, Wagner, R., 1994, *Nature* 372:333-335. Thus, oligonucleotides complementary to either the 5'- or 3'- non-translated, non-coding regions of the HGPRBMY2 shown in SEQ ID NO:13, could be used in an antisense approach to inhibit translation of endogenous HGPRBMY2 mRNA. Oligonucleotides complementary to the 5' untranslated region of the mRNA should include the complement of the AUG start codon. Antisense oligonucleotides complementary to mRNA coding regions are less efficient inhibitors of translation but could be used in accordance with the invention. Whether designed to hybridize to the 5'-, 3'- or coding region of HGPRBMY2 mRNA, antisense nucleic acids should be at least six nucleotides in length, and are preferably oligonucleotides ranging from 6 to about 50 nucleotides in length. In specific aspects the oligonucleotide is at least nucleotides, at least 17 nucleotides, at least 25 nucleotides or at least 50 nucleotides.

Regardless of the choice of target sequence, it is preferred that *in vitro* studies are first performed to quantitate the ability of the antisense oligonucleotide to inhibit gene expression. It is preferred that these studies utilize controls that distinguish between antisense gene inhibition and nonspecific biological effects of oligonucleotides. It is also preferred that these studies compare levels of the target RNA or polypeptide with that of an internal control RNA or polypeptide. Additionally, it is envisioned that results

obtained using the antisense oligonucleotide are compared with those obtained using a control oligonucleotide. It is preferred that the control oligonucleotide is of approximately the same length as the test oligonucleotide and that the nucleotide sequence of the oligonucleotide differs from the antisense sequence no more than is necessary to prevent specific hybridization to the target sequence.

The oligonucleotides can be DNA or RNA or chimeric mixtures or derivatives or modified versions thereof, single-stranded or double-stranded. The oligonucleotide can be modified at the base moiety, sugar moiety, or phosphate backbone, for example, to improve stability of the molecule, hybridization, etc. The oligonucleotide may include other appended groups such as peptides (*e.g.*, for targeting host cell receptors *in vivo*), or agents facilitating transport across the cell membrane (see, *e.g.*, Letsinger et al., 1989, Proc. Natl. Acad. Sci. U.S.A. 86:6553-6556; Lemaitre et al., 1987, Proc. Natl. Acad. Sci. 84:648-652; PCT Publication No. WO88/09810, published Dec. 15, 1988), hybridization-triggered cleavage agents. (See, *e.g.*, Krol et al., 1988, BioTechniques 6:958-976) or intercalating agents. (See, *e.g.*, Zon, 1988, Pharm. Res. 5:539-549). To this end, the oligonucleotide may be conjugated to another molecule, *e.g.*, a peptide, hybridization triggered cross-linking agent, transport agent, hybridization-triggered cleavage agent, etc.

The antisense oligonucleotide may comprise at least one modified base moiety which is selected from the group including but not limited to 5-fluorouracil, 5-bromouracil, 5-chlorouracil, 5-iodouracil, hypoxanthine, xanthine, 4-acetylcytosine, 5-(carboxyhydroxymethyl) uracil, 5-carboxymethylaminomethyl-2-thiouridine, 5-carboxymethylaminomethyluracil, dihydrouracil,  $\beta$ -D-galactosylqueosine, inosine, N6-isopentenyladenine, 1-methylguanine, 1-methylinosine, 2,2-dimethylguanine, 2-methyladenine, 2-methylguanine, 3-methylcytosine, 5-methylcytosine, N6-adenine, 7-methylguanine, 5-methylaminomethyluracil, 5-methoxyaminomethyl-2-thiouracil,  $\beta$ -D-mannosylqueosine, 5'-methoxycarboxymethyluracil, 5-methoxyuracil, 2-methylthio-N6-isopentenyladenine, uracil-5-oxyacetic acid (v), wybutoxosine, pseudouracil, queosine, 2-thiacytosine, 5-methyl-2-thiouracil, 2-thiouracil, 4-thiouracil, 5-methyluracil, uracil-5-oxyacetic acid methylester, uracil-5-oxyacetic acid (v), 5-methyl-2-thiouracil, 3-(3-amino-3-N-2-carboxypropyl) uracil, (acp3)w, and 2,6-diaminopurine. The antisense oligonucleotide may also comprise at least one

modified sugar moiety selected from the group including but not limited to arabinose, 2-fluoroarabinose, xylulose, and hexose.

In yet another embodiment, the antisense oligonucleotide comprises at least one modified phosphate backbone selected from the group consisting of a phosphorothioate, a phosphorodithioate, a phosphoramidothioate, a phosphoramidate, a phosphordiamidate, a methylphosphonate, an alkyl phosphotriester, and a formacetal or analog thereof.

In yet another embodiment, the antisense oligonucleotide is an  $\alpha$ -anomeric oligonucleotide. An  $\alpha$ -anomeric oligonucleotide forms specific double-stranded hybrids with complementary RNA in which, contrary to the usual  $\beta$ -units, the strands run parallel to each other (Gautier et al., 1987, *Nucl. Acids Res.* 15:6625-6641). The oligonucleotide is a 2'-0-methylribonucleotide (Inoue et al., 1987, *Nucl. Acids Res.* 15:6131-6148), or a chimeric RNA-DNA analogue (Inoue et al., 1987, *FEBS Lett.* 215:327-330).

Oligonucleotides of the invention may be synthesized by standard methods known in the art, *e.g.* by use of an automated DNA synthesizer (such as are commercially available from Biosearch, Applied Biosystems, etc.). As examples, phosphorothioate oligonucleotides may be synthesized by the method of Stein et al. (1988, *Nucl. Acids Res.* 16:3209), methylphosphonate oligonucleotides can be prepared by use of controlled pore glass polymer supports (Sarin et al., 1988, *Proc. Natl. Acad. Sci. U.S.A.* 85:7448-7451), etc.

While antisense nucleic acids complementary to the HGPRBMY2 coding region sequence could be used, those complementary to the transcribed untranslated region are most preferred. For example, antisense oligonucleotides having the following sequences can be utilized in accordance with the invention:

- a) 5'-CATGCGGGGCAGCGAGG-3' (SEQ ID NO:30) which is complementary to nucleotides -14 to +3 as shown in SEQ ID NO:13;
- b) 5'-CATGCGGGGCAGCGAGGGCTTCGG-3' (SEQ ID NO:31) which is complementary to nucleotides -20 to +3 in SEQ ID NO:13.

The antisense molecules should be delivered to cells which express HGPRBMY2 *in vivo*, *e.g.*, the heart. A number of methods have been developed for delivering antisense DNA or RNA to cells; *e.g.*, antisense molecules can be injected directly into the tissue site, or modified antisense molecules, designed to target the desired cells (*e.g.*,

antisense linked to peptides or antibodies that specifically bind receptors or antigens expressed on the target cell surface) can be administered systemically.

Alternatively, an antisense nucleic acid is delivered via a recombinant DNA construct in which the antisense oligonucleotide is placed under the control of a strong pol III or pol II promoter. The use of such a construct to transfect target cells in the patient will result in the transcription of sufficient amounts of single stranded RNAs that will form complementary base pairs with the endogenous HGPRBMY2 transcripts and thereby prevent translation of the HGPRBMY2 mRNA. For example, a vector can be introduced *in vivo* such that it is taken up by a cell and directs the transcription of an antisense RNA. Such a vector can remain episomal or become chromosomally integrated, as long as it can be transcribed to produce the desired antisense RNA. Such vectors can be constructed by recombinant DNA technology methods standard in the art.

Vectors can be plasmid, viral, or others known in the art, used for replication and expression in mammalian cells. Expression of the sequence encoding the antisense RNA can be by any promoter known in the art to act in mammalian, preferably human cells. Such promoters can be inducible or constitutive. Such promoters include but are not limited to: the SV40 early promoter region (Bernoist and Chambon, 1981, *Nature* 290:304-310), the promoter contained in the 3' long terminal repeat of Rous sarcoma virus (Yamamoto et al., 1980, *Cell* 22:787-797), the herpes thymidine kinase promoter (Wagner et al., 1981, *Proc. Natl. Acad. Sci. U.S.A.* 78:1441-1445), the regulatory sequences of the metallothionein gene (Brinster et al., 1982, *Nature* 296:39-42), etc. Any type of plasmid, cosmid, YAC or viral vector can be used to prepare the recombinant DNA construct which can be introduced directly into the tissue site; *e.g.*, the heart. Alternatively, viral vectors can be used which selectively infect the desired tissue; (*e.g.*, for heart, herpesvirus vectors may be used), in which case administration may be accomplished by another route (*e.g.*, systemically).

Ribozyme molecules-designed to catalytically cleave HGPRBMY2 mRNA transcripts can also be used to prevent translation of HGPRBMY2mRNA and expression of HGPRBMY2. (See, *e.g.*, PCT International Publication WO90/11364, published Oct. 4, 1990; Sarver et al., 1990, *Science* 247:1222-1225). While ribozymes that cleave mRNA at site specific recognition sequences can be used to destroy HGPRBMY2 mRNAs, the use of hammerhead ribozymes is preferred. Hammerhead ribozymes cleave

mRNAs at locations dictated by flanking regions that form complementary base pairs with the target mRNA. The sole requirement is that the target mRNA have the following sequence of two bases: 5'-UG-3'. The construction and production of hammerhead ribozymes is well known in the art and is described more fully in Haseloff and Gerlach, 1988, *Nature*, 334:585-591. There are hundreds of potential hammerhead ribozyme cleavage sites within the nucleic acid sequence of human HGPRBMY2 cDNA. Preferably the ribozyme is engineered so that the cleavage recognition site is located near the 5' end of the HGPRBMY2 mRNA; *i.e.*, to increase efficiency and minimize the intracellular accumulation of non-functional mRNA transcripts. For example, hammerhead ribozymes can be utilized in accordance with the invention.

The ribozymes of the present invention also include RNA endoribonucleases (hereinafter "Cech-type ribozymes") such as the one which occurs naturally in *Tetrahymena Thermophila* (known as the IVS, or L-19 IVS RNA) and which has been extensively described by Thomas Cech and collaborators (Zaug, et al., 1984, *Science*, 224:574-578; Zaug and Cech, 1986, *Science*, 231:470-475; Zaug, et al., 1986, *Nature*, 324:429-433; published International patent-application No. WO 88/04300 by University Patents Inc.; Been and Cech, 1986, *Cell*, 47:207-216). The Cech-type ribozymes have an eight base pair active site which hybridizes to a target RNA sequence whereafter cleavage of the target RNA takes place. The invention encompasses those Cech-type ribozymes which target eight base-pair active site sequences that are present in HGPRBMY2.

As in the antisense approach, the ribozymes can be composed of modified oligonucleotides (*e.g.* for improved stability, targeting, etc.) and should be delivered to cells which express the HGPRBMY2 *in vivo*, *e.g.*, heart. A preferred method of delivery involves using a DNA construct "encoding" the ribozyme under the control of a strong constitutive pol III or pol II promoter, so that transfected cells will produce sufficient quantities of the ribozyme to destroy endogenous HGPRBMY2 messages and inhibit translation. Because ribozymes unlike antisense molecules, are catalytic, a lower intracellular concentration is required for efficiency.

Endogenous HGPRBMY2 gene expression can also be reduced by inactivating or "knocking out" the HGPRBMY2 gene or its promoter using targeted homologous recombination (*e.g.*, see Smithies et al., 1985, *Nature* 317:230-234; Thomas & Capecchi, 1987, *Cell* 51:503-512; Thompson et al., 1989 *Cell* 5:313-321; each of which is

incorporated by reference herein in its entirety). For example, a mutant, non-functional HGPRBMY2 flanked by DNA homologous to the endogenous HGPRBMY2 gene locus, coding or regulatory, can be used with or without a selectable marker and/or a negative selectable marker to transfect cells that express HGPRBMY2 *in vivo*. Insertion of the DNA construct, via targeted homologous recombination, results in inactivation of the HGPRBMY2 gene. Such approaches are particularly suited in the agricultural field where modifications to ES (embryonic stem) cells can be used to generate animal offspring with an inactive HGPRBMY2 (*e.g.*, see Thomas & Capecchi 1987 and Thompson 1989, *supra*). However this approach can be adapted for use in humans provided the recombinant DNA constructs are directly administered or targeted to the required site *in vivo* using appropriate viral vectors, *e.g.*, herpes virus vectors for delivery to tissue; *e.g.*, heart.

Alternatively, endogenous HGPRBMY2 gene expression can be reduced by targeting deoxyribonucleotide sequences complementary to the regulatory region of the HGPRBMY2 gene (*i.e.*, the HGPRBMY2 promoter and/or enhancers) to form triple helical structures that prevent transcription of the HGPRBMY2 gene in target cells in the body. (See generally, Helene, C. 1991, *Anticancer Drug Des.*, 6(6):569-84; Helene, C., et al., 1992, *Ann, N.Y. Accad. Sci.*, 660:27-36; and Maher, L. J., 1992, *Bioassays* 14(12):807-15).

In yet another embodiment of the invention, the activity of HGPRBMY2 can be reduced using a “dominant negative” approach to prevent or treat heart failure. To this end, constructs which encode defective HGPRBMY2 can be used in gene therapy approaches to diminish the activity of the HGPRBMY2 in appropriate target cells. For example, nucleic acid sequences that direct host cell expression of HGPRBMY2 in which the CD is deleted or mutated can be introduced into cells in the heart (either by *in vivo* or *ex vivo* gene therapy methods described above). Alternatively, targeted homologous recombination can be utilized to introduce such deletions or mutations into the subject’s endogenous HGPRBMY2 gene in the heart. The engineered cells will express non-functional receptors (*i.e.*, an anchored receptor that is capable of binding its natural ligand, but incapable of signal transduction). Such engineered cells present in the heart should demonstrate a diminished response to the endogenous agonist or antagonist ligand, resulting in heart failure.

With respect to an increase in the level of normal HGPRBMY2 gene expression and/or HGPRBMY2 gene product activity, HGPRBMY2 nucleic acid sequences can be utilized for the treatment of cardiovascular disorders, including congestive heart failure. Where the cause of congestive heart failure is a defective HGPRBMY2, treatment can be administered, for example, in the form of gene replacement therapy.

In another embodiment, the expression characteristics of an endogenous gene (e.g., HGPRBMY2 genes) within a cell, cell line or microorganism may be modified by inserting a DNA regulatory element heterologous to the endogenous gene of interest into the genome of a cell, stable cell line or cloned microorganism such that the inserted regulatory element is operatively linked with the endogenous gene (e.g., HGPRBMY2 genes) and controls, modulates or activates. For example, endogenous HGPRBMY2 genes which are normally "transcriptionally silent", i.e., a HGPRBMY2 genes which is normally not expressed, or are expressed only at very low levels in a cell line or microorganism, may be activated by inserting a regulatory element which is capable of promoting the expression of a normally expressed gene product in that cell line or microorganism. Alternatively, transcriptionally silent, endogenous HGPRBMY2 genes may be activated by insertion of a promiscuous regulatory element that works across cell types.

A heterologous regulatory element may be inserted into a stable cell line or cloned microorganism, such that it is operatively linked with and activates expression of endogenous HGPRBMY2 genes, using techniques, such as targeted homologous recombination, which are well known to those of skill in the art, and described e.g., in Chappel, U.S. Patent No. 5,272,071; PCT publication No. WO 91/06667, published May 16, 1991; Skoultschi U.S. Patent No. 5,981,214; Treco *et al* U.S. Patent No. 5,968,502 and PCT publication No. WO 94/12650, published June 9, 1994. Alternatively, non-targeted e.g., non-homologous recombination techniques which are well-known to those of skill in the art and described, e.g., in PCT publication No. WO 99/15650, published April 1, 1999, may be used.

Specifically, one or more copies of a normal HGPRBMY2 gene or a portion of the HGPRBMY2 gene that directs the production of an HGPRBMY2 gene product exhibiting normal function, may be inserted into the appropriate cells within a patient or animal subject, using vectors which include, but are not limited to adenovirus,

adeno-associated virus, retrovirus and herpes virus vectors, in addition to other particles that introduce DNA into cells, such as liposomes.

Because the HGPRBMY2 gene is expressed in the heart and thymus, such gene replacement therapy techniques should be capable of delivering HGPRBMY2 gene sequences to these cell types within patients. Thus, the techniques for delivery of the HGPRBMY2 gene sequences should be designed to readily involve direct administration of such HGPRBMY2 gene sequences to the site of the cells in which the HGPRBMY2 gene sequences are to be expressed. Alternatively, targeted homologous recombination can be utilized to correct the defective endogenous HGPRBMY2 gene in the appropriate tissue; *e.g.*, heart. In animals, targeted homologous recombination can be used to correct the defect in ES cells in order to generate offspring with a corrected trait.

Additional methods which may be utilized to increase the overall level of HGPRBMY2 gene expression and/or HGPRBMY2 activity include the introduction of appropriate HGPRBMY2-expressing cells, preferably autologous cells, into a patient at positions and in numbers which are sufficient to ameliorate the symptoms of cardiovascular disorders, including congestive heart failure. Such cells may be either recombinant or non-recombinant. Among the cells which can be administered to increase the overall level of HGPRBMY2 gene expression in a patient are normal cells, preferably heart cells, cells which express the HGPRBMY2 gene. The cells can be administered at the anatomical site in the body, or as part of a tissue graft located at a different site in the body. Such cell-based gene therapy techniques are well known to those skilled in the art, see, *e.g.*, Anderson, et al., 5,399,349; Mulligan & Wilson, U.S. Pat. No. 5,460,959.

Finally, compounds, identified in the assays described above, that stimulate or enhance the signal transduced by activated HGPRBMY2, *e.g.*, by activating downstream signalling polypeptides in the HGPRBMY2 cascade and thereby by-passing the defective HGPRBMY2, can be used to ameliorate cardiovascular disease. The formulation and mode of administration will depend upon the physico-chemical properties of the compound.

## 5.7. Pharmaceutical Preparations and Methods of Administration

The compounds that are determined to affect HGPRBMY1 gene expression or HGPRBMY1 activity can be administered to a patient at therapeutically effective doses to treat or ameliorate bone marrow or spleen disorders. A therapeutically effective dose refers to that amount of the compound sufficient to result in amelioration of symptoms of immune disorders.

The compounds that are determined to affect HGPRBMY2 gene expression or HGPRBMY2 activity can be administered to a patient at therapeutically effective doses to treat or ameliorate heart disorders. A therapeutically effective dose refers to that amount of the compound sufficient to result in amelioration of symptoms of cardiovascular or neural disorders.

### 5.7.1. Effective Dose

Toxicity and therapeutic efficacy of such compounds can be determined by standard pharmaceutical procedures in cell cultures or experimental animals, *e.g.*, for determining the LD<sub>50</sub> (the dose lethal to 50% of the population) and the ED<sub>50</sub> (the dose therapeutically effective in 50% of the population). The dose ratio between toxic and therapeutic effects is the therapeutic index and it can be expressed as the ratio LD<sub>50</sub> /ED<sub>50</sub>. Compounds which exhibit large therapeutic indices are preferred.

While compounds that exhibit toxic side effects may be used, care should be taken to design a delivery system that targets such compounds to the site of affected tissue in order to minimize potential damage to uninfected cells and, thereby, reduce side effects.

The data obtained from the cell culture assays and animal studies can be used in formulating a range of dosage for use in humans. The dosage of such compounds lies preferably within a range of circulating concentrations that include the ED<sub>50</sub> with little or no toxicity. The dosage may vary within this range depending upon the dosage form employed and the route of administration utilized. For any compound used in the method of the invention, the therapeutically effective dose can be estimated initially from cell culture assays. A dose may be formulated in animal models to achieve a circulating plasma concentration range that includes the IC<sub>50</sub> (*i.e.*, the concentration of the test

compound which achieves a half-maximal inhibition of symptoms) as determined in cell culture. Such information can be used to more accurately determine useful doses in humans. Levels in plasma may be measured, for example, by high performance liquid chromatography.

#### **5.7.2. Formulations and Use**

Pharmaceutical compositions for use in accordance with the present invention may be formulated in conventional manner using one or more physiologically acceptable carriers or excipients.

Thus, the compounds and their physiologically acceptable salts and solvates may be formulated for administration by inhalation or insufflation (either through the mouth or the nose) or oral, buccal, parenteral or rectal administration.

For oral administration, the pharmaceutical compositions may take the form of, for example, tablets or capsules prepared by conventional means with pharmaceutically acceptable excipients such as binding agents (*e.g.*, pregelatinised maize starch, polyvinylpyrrolidone or hydroxypropyl methylcellulose); fillers (*e.g.*, lactose, microcrystalline cellulose or calcium hydrogen phosphate); lubricants (*e.g.*, magnesium stearate, talc or silica); disintegrants (*e.g.*, potato starch or sodium starch glycolate); or wetting agents (*e.g.*, sodium lauryl sulphate). The tablets may be coated by methods well known in the art. Liquid preparations for oral administration may take the form of, for example, solutions, syrups or suspensions, or they may be presented as a dry product for constitution with water or other suitable vehicle before use. Such liquid preparations may be prepared by conventional means with pharmaceutically acceptable additives such as suspending agents (*e.g.*, sorbitol syrup, cellulose derivatives or hydrogenated edible fats); emulsifying agents (*e.g.*, lecithin or acacia); non-aqueous vehicles (*e.g.*, almond oil, oily esters, ethyl alcohol or fractionated vegetable oils); and preservatives (*e.g.*, methyl or propyl-p-hydroxybenzoates or sorbic acid). The preparations may also contain buffer salts, flavoring, coloring and sweetening agents as appropriate.

Preparations for oral administration may be suitably formulated to give controlled release of the active compound.

For buccal administration the compositions may take the form of tablets or lozenges formulated in conventional manner.

For administration by inhalation, the compounds for use according to the present invention are conveniently delivered in the form of an aerosol spray presentation from pressurized packs or a nebulizer, with the use of a suitable propellant, *e.g.*, dichlorodifluoromethane, trichlorofluoromethane, dichlorotetrafluoroethane, carbon dioxide or other suitable gas. In the case of a pressurized aerosol the dosage unit may be determined by providing a valve to deliver a metered amount. Capsules and cartridges of *e.g.* gelatin for use in an inhaler or insufflator may be formulated containing a powder mix of the compound and a suitable powder base such as lactose or starch.

The compounds may be formulated for parenteral administration by injection, *e.g.*, by bolus injection or continuous infusion.

Formulations for injection may be presented in unit dosage form, *e.g.*, in ampoules or in multi-dose containers, with an added preservative. The compositions may take such forms as suspensions, solutions or emulsions in oily or aqueous vehicles, and may contain formulatory agents such as suspending, stabilizing and/or dispersing agents. Alternatively, the active ingredient may be in powder form for constitution with a suitable vehicle, *e.g.*, sterile pyrogen-free water, before use.

The compounds may also be formulated in rectal compositions such as suppositories or retention enemas, *e.g.*, containing conventional suppository bases such as cocoa butter or other glycerides.

In addition to the formulations described previously, the compounds may also be formulated as a depot preparation. Such long acting formulations may be administered by implantation (for example subcutaneously or intramuscularly) or by intramuscular injection. Thus, for example, the compounds may be formulated with suitable polymeric or hydrophobic materials (for example as an emulsion in an acceptable oil) or ion exchange resins, or as sparingly soluble derivatives, for example, as a sparingly soluble salt.

The compositions may, if desired, be presented in a pack or dispenser device which may contain one or more unit dosage forms containing the active ingredient. The pack may for example comprise metal or plastic foil, such as a blister pack. The pack or dispenser device may be accompanied by instructions for administration.

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## EXAMPLES

### **Example 1 – HGPRBMY1 Bioinformatics Analysis**

G-protein coupled receptor sequences were used as a probe to search the Incyte and public domain EST databases. All the G-protein coupled receptor sequences available at the GPCRdb GPCR Database (<http://www.gpcr.org/7tm>) were used as queries. The search program used was gapped BLAST (Altschul, et al., 1997, *Nucleic Acids Res* 25:3389-3402). The top EST hits from the BLAST results were searched back against the non-redundant polypeptide and patent sequence databases. From this analysis, ESTs encoding a potential novel GPCRs were identified based on sequence homology. The public domain EST (ATCC® CloneID: 145375) was selected as potential novel GPCR candidate, HGPRBMY1 for subsequent analysis.

This EST was sequenced over its full length and was shown to contain a coding region bearing distinctive characteristics of a G-protein coupled receptor (GPCR). More

specifically, the complete polypeptide sequence of HGPRBMY1 was analyzed for potential transmembrane domains. The TMPRED program was used for transmembrane prediction (K Hofmann and W Stoffel, 1993, Biol. Chem. Hoppe-Seyler 347:166). The program predicted seven transmembrane domains and the predicted domains match with the predicted transmembrane domains of related GPCRs at the sequence level. Based on sequence, structure and known GPCR signature sequences, the orphan polypeptide, HGPRBMY1, is likely a novel human GPCR.

#### **Example 2 - Cloning of the Novel Human GPCR HGPRBMY1**

A PCR primer pair, designed from the DNA sequence of ATCC® clone was used to amplify a piece of DNA from the same clone in which the antisense strand of the amplified fragment was biotinylated on the 3' end. This biotinylated piece of double stranded DNA was denatured and incubated with a mixture of single-stranded covalently closed circular cDNA libraries which contain DNA corresponding to the sense strand.

Hybrids between the biotinylated DNA and the circular cDNA were captured on streptavidin magnetic beads. Upon thermal release of the cDNA from the biotinylated DNA, the single stranded cDNA was converted into double strands using a primer homologous to a sequence on the cDNA cloning vector. The double stranded cDNA was introduced into *E. coli* by electroporation and the resulting colonies were screen by PCR, using the original primer pair, to identify the proper cDNA.

#### **Example 3 - Expression profiling of novel human GPCR, HGPRBMY1**

A PCR primer was designed from the ATCC® clone and was used to measure the steady state levels of mRNA by quantitative PCR. The sequence of the primer pair was as follows:

5'-GATCCCCGTCGGTCATCTT-3' (SEQ ID NO:3)

5'-GGTCACCACGTTGCAAAGC-3' (SEQ ID NO:4)

Briefly, first strand cDNA was made from commercially available mRNA. The relative amount of cDNA used in each assay was determined by performing a parallel experiment using a primer pair for a gene expressed in equal amounts in all tissues, cyclophilin. The cyclophilin primer pair detected small variations in the amount of cDNA

in each sample and these data were used for normalization of the data obtained with the primer pair for this gene. The PCR data was converted into a relative assessment of the difference in transcript abundance amongst the tissues tested and the data is presented in Figure 5.

Transcripts corresponding to the orphan GPCR, HGPRBMY1, are expressed highly in bone marrow and spleen, and to a lesser extent in the thymus.

**Example 4 - Complementary Polynucleotides And Association of HGPRBMY1 To Cell Cycle and Apoptosis Regulation**

Antisense molecules or nucleic acid sequences complementary to the HGPRBMY1 protein-encoding sequence, or any part thereof, is used to decrease or to inhibit the expression of naturally occurring HGPRBMY1. Although the use of antisense or complementary oligonucleotides comprising about 15 to 35 base-pairs is described, essentially the same procedure is used with smaller or larger nucleic acid sequence fragments. An oligonucleotide based on the coding sequence of HGPRBMY1 protein, as shown in Figure 1, or as depicted in SEQ ID NO:1, for example, is used to inhibit expression of naturally occurring HGPRBMY1. The complementary oligonucleotide is typically designed from the most unique 5' sequence and is used either to inhibit transcription by preventing promoter binding to the coding sequence, or to inhibit translation by preventing the ribosome from binding to the HGPRBMY1 protein-encoding transcript, among others. However, other regions may also be targeted.

Using an appropriate portion of the signal and 5' sequence of SEQ ID NO:1, an effective antisense oligonucleotide includes any of about 15-35 nucleotides spanning the region which translates into the signal or 5' coding sequence, among other regions, of the polypeptide as shown in Figure 2 (SEQ ID NO:2). Appropriate oligonucleotides are designed using OLIGO 4.06 software and the HGPRBMY1 protein coding sequence (SEQ ID NO:1). Preferred oligonucleotides are deoxynucleotide-, or chimeric deoxynucleotide/ribonucleotide-based and are provided below. The oligonucleotides were synthesized using chemistry essentially as described in U.S. Patent No. 5,849,902; which is hereby incorporated herein by reference in its entirety.

ID#	Sequence
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15214	ACAGGAUGCAACGCUUAAGUCGACG (SEQ ID NO:5)
15215	AGAUUUGGAAAGGCAACACGCGUGGC (SEQ ID NO:6)
15216	CUUGAGGACGUCGAAGCAGGUGAUG (SEQ ID NO:7)
15217	GCCUCCUCCGUGCGAACAGCUUGA (SEQ ID NO:8)
15218	CUGAUACAGGUCAUGGUGAGGAUGC (SEQ ID NO:9)

The HGPRBMY1 polypeptide has been shown to be involved in the regulation of mammalian cell cycle pathways. Subjecting cells with an effective amount of a pool of all five of the above antisense oligonucleotides (SEQ ID NO:5 thru 9) resulted in a significant increase in p27 expression/activity providing convincing evidence that HGPRBMY1 at least regulates the activity and/or expression of p27 either directly, or indirectly. Moreover, the results suggest that HGPRBMY1 is involved in the negative regulation of p27 activity and/or expression, either directly or indirectly. The p27 assay used is described below and was based upon the analysis of p27 activity as a downstream marker for proliferative signal transduction events.

Moreover, the HGPRBMY1 polypeptide has also been shown to be involved in the regulation of mammalian NF- $\kappa$ B and apoptosis pathways. Subjecting cells with an effective amount of a pool of all five of the above antisense oligonucleotides (SEQ ID NO:5 thru 9) resulted in a significant increase in I $\kappa$ B $\alpha$  expression/activity providing convincing evidence that HGPRBMY1 at least regulates the activity and/or expression of I $\kappa$ B $\alpha$  either directly, or indirectly. Moreover, the results suggest that HGPRBMY1 is involved in the negative regulation of NF- $\kappa$ B/I $\kappa$ B $\alpha$  activity and/or expression, either directly or indirectly. The I $\kappa$ B $\alpha$  assay used is described below and was based upon the analysis of I $\kappa$ B $\alpha$  activity as a downstream marker for proliferative signal transduction events.

Based upon the regulation of p27 and I $\kappa$ B, antagonists directed against HGPRBMY1 would be useful for upregulating P27 and I $\kappa$ B, which would be beneficial to cancer patients by stopping proliferation and inducing apoptosis of a cell comprising a tumor.

*Transfection of post-quiescent A549 cells With AntiSense Oligonucleotides.***Materials needed:**

- A549 cells maintained in DMEM with high glucose (Gibco-BRL) supplemented with 10% Fetal Bovine Serum, 2mM L-Glutamine, and 1X penicillin/streptomycin.
- Opti-MEM (Gibco-BRL)
- Lipofectamine 2000 (Invitrogen)
- Antisense oligomers (Sequitur)
- Polystyrene tubes.
- Tissue culture treated plates.

**Quiescent cells were prepared as follows:**

Day 0: 300, 000 A549 cells were seeded in a T75 tissue culture flask in 10 ml of A549

media, and incubated in at 37°C, 5% CO<sub>2</sub> in a humidified incubator for 48 hours.

Day 2: The T75 flasks were rocked to remove any loosely adherent cells, and the A549 growth media removed and replenished with 10 ml of fresh A549 media. The cells were cultured for six days without changing the media to create a quiescent cell population.

Day 8: Quiescent cells were plated in multi-well format and transfected with antisense oligonucleotides.

**A549 cells were transfected according to the following:**

1. Trypsinize T75 flask containing quiescent population of A549 cells.
2. Count the cells and seed 24-well plates with 60K quiescent A549 cells per well.
3. Allow the cells to adhere to the tissue culture plate (approximately 4 hours).
4. Transfect the cells with antisense and control oligonucleotides according to the following:

- a. A 10X stock of lipofectamine 2000 (10 ug/ml is 10X) was prepared, and diluted lipid was allowed to stand at RT for 15 minutes.  
Stock solution of lipofectamine 2000 was 1 mg/ml.  
10 X solution for transfection was 10 ug/ml.  
To prepare 10X solution, dilute 10 ul of lipofectamine 2000 stock per 1 ml of Opti-MEM (serum free media).
- b. A 10X stock of each oligomer was prepared to be used in the transfection.  
Stock solutions of oligomers were at 100 uM in 20 mM HEPES, pH 7.5.  
10X concentration of oligomer was 0.25 uM.  
To prepare the 10X solutions, dilute 2.5 ul of oligomer per 1 ml of Opti-MEM.
- c. Equal volumes of the 10X lipofectamine 2000 stock and the 10X oligomer solutions were mixed well, and incubated for 15 minutes at RT to allow complexation of the oligomer and lipid. The resulting mixture was 5X.
- d. After the 15 minute complexation, 4 volumes of full growth media was added to the oligomer/lipid complexes (solution was 1X).
- e. The media was aspirated from the cells, and 0.5 ml of the 1X oligomer/lipid complexes added to each well.
- f. The cells were incubated for 16-24 hours at 37°C in a humidified CO<sub>2</sub> incubator.
- g. Cell pellets were harvested for RNA isolation and TaqMan analysis of downstream marker genes.

#### *TaqMan Reactions – p27 Reactions*

Quantitative RT-PCR analysis was performed on total RNA preps that had been treated with DNaseI or poly A selected RNA. The Dnase treatment may be performed using methods known in the art, though preferably using a Qiagen RNeasy kit to purify the RNA samples, wherein DNase I treatment is performed on the column.

Briefly, a master mix of reagents was prepared according to the following table:

Dnase I Treatment

<u>Reagent</u>	<u>Per rxn (in uL)</u>
10x Buffer	2.5
Dnase I (1 unit/uL @ 1 unit per ug 2 sample)	
DEPC H <sub>2</sub> O	0.5
RNA sample @ 0.1 ug/uL	20
	(2-3 ug total)
Total	25

Next, 5 uL of master mix was aliquoted per well of a 96-well PCR reaction plate (PE part # N801-0560). RNA samples were adjusted to 0.1 ug/uL with DEPC treated H<sub>2</sub>O (if necessary), and 20 uL was added to the aliquoted master mix for a final reaction volume of 25 uL.

The wells were capped using strip well caps (PE part # N801-0935), placed in a plate, and briefly spun in a centrifuge to collect all volume in the bottom of the tubes. Generally, a short spin up to 500rpm in a Sorvall RT is sufficient.

The plates were incubated at 37°C for 30 mins. Then, an equal volume of 0.1mM EDTA in 10mM Tris was added to each well, and heat inactivated at 70°C for 5 min. The plates were stored at -80°C upon completion.

RT reaction

A master mix of reagents was prepared according to the following table:

RT reaction

<u>Reagent</u>	<u>1</u> <u>x'n (in uL)</u>	<u>No RT</u> <u>x'n (in uL)</u>
10x RT buffer	5	2.5
MgCl <sub>2</sub>	11	5.5

DNTP mixture	10	5
Random Hexamers	2.5	1.25
Rnase inhibitors	1.25	0.625
RT enzyme	1.25	-
Total RNA 500ng (100ng 19.0 max no RT)		10.125 max
DEPC H <sub>2</sub> O	-	-
Total	50uL	25uL

Samples were adjusted to a concentration so that 500ng of RNA was added to each RT rx'n (100ng for the no RT). A maximum of 19 ul can be added to the RT rx'n mixture (10.125 ul for the no RT.) Any remaining volume up to the maximum values was filled with DEPC treated H<sub>2</sub>O, so that the total reaction volume was 50 ul (RT) or 25 ul (no RT).

On a 96-well PCR reaction plate (PE part # N801-0560), 37.5 ul of master mix was aliquoted (22.5 ul of no RT master mix), and the RNA sample added for a total reaction volume of 50ul (25 ul, no RT). Control samples were loaded into two or even three different wells in order to have enough template for generation of a standard curve.

The wells were capped using strip well caps (PE part # N801-0935), placed in a plate, and spin briefly in a centrifuge to collect all volume in the bottom of the tubes. Generally, a short spin up to 500rpm in a Sorvall RT is sufficient.

For the RT-PCR reaction, the following thermal profile was used:

- 25°C for 10 min
- 48°C for 30 min
- 95°C for 5 min
- 4°C hold (for 1 hour)
- Store plate @-20°C or lower upon completion.

*TaqMan reaction (Template comes from RT plate.)*

A master mix was prepared according to the following table:

TaqMan reaction (per well)

<u>Reagent</u>	<u>Per Rx'n (in uL)</u>
TaqMan Master Mix	4.17
100 uM Probe (SEQ ID NO:12)	.025
100 uM Forward primer (SEQ ID NO:10)	.05
100 uM Reverse primer (SEQ ID NO:11)	.05
Template	-
DEPC H <sub>2</sub> O	18.21
Total	22.5

The primers used for the RT-PCR reaction is as follows:

P27 primer and probes:

Forward Primer: CCCGGTGGACCACGAA (SEQ ID NO:10)

Reverse Primer: GGCTCGCCTTCCATGTC (SEQ ID NO:11)

TaqMan Probe: AACCCGGGACTTGGAGAAGCACTGC (SEQ ID NO:12)

*TaqMan Reactions – I kB Reactions*

Quantitative RT-PCR analysis was performed on total RNA preps that had been treated with DNaseI or poly A selected RNA. The Dnase treatment may be performed using methods known in the art, though preferably using a Qiagen RNeasy kit to purify the RNA samples, wherein DNase I treatment is performed on the column.

Briefly, a master mix of reagents was prepared according to the following table:

Dnase I Treatment

<u>Reagent</u>	<u>Per rxn (in uL)</u>
10x Buffer	2.5
Dnase I (1 unit/uL @ 1 unit per ug 2 sample)	
DEPC H <sub>2</sub> O	0.5
RNA sample @ 0.1 ug/uL	20
	(2-3 ug total)
Total	25

Next, 5 uL of master mix was aliquoted per well of a 96-well PCR reaction plate (PE part # N801-0560). RNA samples were adjusted to 0.1 ug/uL with DEPC treated H<sub>2</sub>O (if necessary), and 20 uL was added to the aliquoted master mix for a final reaction volume of 25 uL.

The wells were capped using strip well caps (PE part # N801-0935), placed in a plate, and briefly spun in a centrifuge to collect all volume in the bottom of the tubes. Generally, a short spin up to 500rpm in a Sorvall RT is sufficient

The plates were incubated at 37°C for 30 mins. Then, an equal volume of 0.1mM EDTA in 10mM Tris was added to each well, and heat inactivated at 70°C for 5 min. The plates were stored at -80°C upon completion.

*RT reaction*

A master mix of reagents was prepared according to the following table:

RT reaction

<u>Reagent</u>	<u>1</u> <u>x'n (in ul)</u>	<u>No RT</u> <u>x'n (in ul)</u>
10x RT buffer	5	2.5
MgCl <sub>2</sub>	11	5.5
DNTP mixture	10	5
Random Hexamers	2.5	1.25
Rnase inhibitors	1.25	0.625
RT enzyme	1.25	-
Total RNA 500ng (100ng 19.0 max no RT )	19.0	10.125 max
DEPC H <sub>2</sub> O	-	-
Total	50uL	25uL

Samples were adjusted to a concentration so that 500ng of RNA was added to each RT rx'n (100ng for the no RT). A maximum of 19 ul can be added to the RT rx'n mixture (10.125 ul for the no RT.) Any remaining volume up to the maximum values was filled with DEPC treated H<sub>2</sub>O, so that the total reaction volume was 50 ul (RT) or 25 ul (no RT).

On a 96-well PCR reaction plate (PE part # N801-0560), 37.5 ul of master mix was aliquoted (22.5 ul of no RT master mix), and the RNA sample added for a total reaction volume of 50ul (25 ul, no RT). Control samples were loaded into two or even three different wells in order to have enough template for generation of a standard curve.

The wells were capped using strip well caps (PE part # N801-0935), placed in a plate, and spin briefly in a centrifuge to collect all volume in the bottom of the tubes. Generally, a short spin up to 500rpm in a Sorvall RT is sufficient.

For the RT-PCR reaction, the following thermal profile was used:

- 25°C for 10 min
- 48°C for 30 min
- 95°C for 5 min

- 4°C hold (for 1 hour)
- Store plate @-20°C or lower upon completion.

*TaqMan reaction (Template comes from RT plate.)*

A master mix was prepared according to the following table:

TaqMan reaction (per well)

<u>Reagent</u>	<u>Per Rx'n (in uL)</u>	
TaqMan Master Mix	4.17	
100 uM Probe	.025	
(SEQ ID NO:15)		
100 uM	.05	
Forward primer (SEQ ID NO:13)		
100 uM	.05	
Reverse primer (SEQ ID NO:14)		
Template	-	
DEPC H <sub>2</sub> O	18.21	
Total	22.5	

The primers used for the RT-PCR reaction is as follows:

IkB primer and probes:

Forward Primer: GAGGATGAGGAGAGCTATGACACA (SEQ ID NO:13)

Reverse Primer: CCCTTTGCACTCATAACGTCAG (SEQ ID NO:14)

TaqMan Probe: AACACACAGTCATCATAGGGCAGCTCGT (SEQ ID NO:15)

Using a Gilson P-10 repeat pipetter, 22.5  $\mu$ l of master mix was aliquoted per well of a 96-well optical plate. Then, using P-10 pipetter, 2.5  $\mu$ l of sample was added to individual wells. Generally, RT samples are run in triplicate with each primer/probe set used, and no RT samples are run once and only with one primer/probe set, often gapdh (or other internal control).

A standard curve is then constructed and loaded onto the plate. The curve has five points plus one no template control (NTC, =DEPC treated H<sub>2</sub>O). The curve was made with a high point of 50 ng of sample (twice the amount of RNA in unknowns), and successive samples of 25, 10, 5, and 1 ng. The curve was made from a control sample(s) (see above).

The wells were capped using optical strip well caps (PE part # N801-0935), placed in a plate, and spun in a centrifuge to collect all volume in the bottom of the tubes. Generally, a short spin up to 500rpm in a Sorvall RT is sufficient.

Plates were loaded onto a PE 5700 sequence detector making sure the plate is aligned properly with the notch in the upper right hand corner. The lid was tightened down and run using the 5700 and 5700 quantitation program and the SYBR probe using the following thermal profile:

- 50°C for 2 min
- 95°C for 10 min
- and the following for 40 cycles:
  - 95°C for 15 sec
  - 60°C for 1 min
  - Change the reaction volume to 25ul.

Once the reaction was complete, a manual threshold of around 0.1 was set to minimize the background signal. Additional information relative to operation of the

GeneAmp 5700 machine may be found in reference to the following manuals: "GeneAmp 5700 Sequence Detection System Operator Training CD"; and the "User's Manual for 5700 Sequence Detection System"; available from Perkin-Elmer and hereby incorporated by reference herein in their entirety.

#### **Example 5 – HGPRBMY2 Bioinformatics Analysis**

G-protein coupled receptor sequences were used as a probe to search the Incyte and public domain EST databases. All the G-protein coupled receptor sequences available at the GPCRdb GPCR Database (<http://www.gpcr.org/7tm>) were used as queries. The search program used was gapped BLAST (Altschul, et al., 1997, Nucleic Acids Res 25:3389-3402). The top EST hits from the BLAST results were searched back against the non-redundant polypeptide and patent sequence databases. From this analysis, ESTs encoding a potential novel GPCRs were identified based on sequence homology. The public domain EST (ATCC CloneID: 3293096) was selected as potential novel GPCR candidate, HGPRBMY2 for subsequent analysis.

This EST was sequenced and the full-length clone of this GPCR was obtained using the EST sequence information. The complete polypeptide sequence of HGPRBMY2 was analyzed for potential transmembrane domains. The TMPRED program was used for transmembrane prediction (K Hofmann and W Stoffel, 1993, Biol. Chem. Hoppe-Seyler 347:166). The program predicted seven transmembrane domains and the predicted domains match with the predicted transmembrane domains of related GPCRs at the sequence level. Based on sequence, structure and known GPCR signature sequences, the orphan polypeptide, HGPRBMY2, is likely a novel human GPCR.

#### **Example 6 - Cloning of the Novel Human GPCR HGPRBMY2**

A PCR primer pair, designed from the DNA sequence of ATCC clone was used to amplify a piece of DNA from the same clone in which the antisense strand of the amplified fragment was biotinylated on the 3' end. This biotinylated piece of double stranded DNA was denatured and incubated with a mixture of single-stranded covalently closed circular cDNA libraries which contain DNA corresponding to the sense strand.

Hybrids between the biotinylated DNA and the circular cDNA were captured on streptavidin magnetic beads. Upon thermal release of the cDNA from the biotinylated

DNA, the single stranded cDNA was converted into double strands using a primer homologous to a sequence on the cDNA cloning vector. The double stranded cDNA was introduced into *E. coli* by electroporation and the resulting colonies were screen by PCR, using the original primer pair, to identify the proper cDNA.

**Example 7 - Expression profiling of novel human GPCR, HGPRBMY2**

A PCR primer was designed from the ATCC clone and was used to measure the steady state levels of mRNA by quantitative PCR. The sequence of the primer pair was as follows:

5'-TTTCTGGATCGTCAGCTTGCT- 3'(SEQ ID NO:15)

5'-ACAGGGCTGGTCCACTCTTCT-3'(SEQ ID NO:16)

Briefly, first strand cDNA was made from commercially available mRNA. The relative amount of cDNA used in each assay was determined by performing a parallel experiment using a primer pair for a gene expressed in equal amounts in all tissues, cyclophilin. The cyclophilin primer pair detected small variations in the amount of cDNA in each sample and these data were used for normalization of the data obtained with the primer pair for this gene. The PCR data was converted into a relative assessment of the difference in transcript abundance amongst the tissues tested and the data is presented in Figure 10.

Transcripts corresponding to the orphan GPCR, HGPRBMY2, are expressed highly in heart, testes and to a lesser degree in thymus.

**Example 8 – Method Of Assessing The Expression Profile Of The Novel HGPRBMY2 Polypeptides Of The Present Invention Using Expanded mRNA Tissue and Cell Sources**

Total RNA from tissues was isolated using the Trizol protocol (Invitrogen) and quantified by determining its absorbance at 260nM. An assessment of the 18s and 28s ribosomal RNA bands was made by denaturing gel electrophoresis to determine RNA integrity.

The specific sequence to be measured was aligned with related genes found in GenBank to identity regions of significant sequence divergence to maximize primer and

probe specificity. Gene-specific primers and probes were designed using the ABI primer 5 express software to amplify small amplicons (150 base pairs or less) to maximize the likelihood that the primers function at 100% efficiency. All primer/probe sequences were searched against Public Genbank databases to ensure target specificity. Primers and probes were obtained from ABI.

10 For HGPRBMY2, the primer probe sequences were as follows

Forward Primer 5' - CACCAACCGAAGGGCTTC -3' (SEQ ID NO:25)

Reverse Primer 5' - CCACATGGGTGATCCTACGAT -3' (SEQ ID NO:26)

15 TaqMan Probe 5' - ACTGCCACCAGCCAGACCACACCTA -3' (SEQ ID NO:27)

**DNA contamination**

To access the level of contaminating genomic DNA in the RNA, the RNA was 20 divided into 2 aliquots and one half was treated with Rnase-free Dnase (Invitrogen). Samples from both the Dnase-treated and non-treated were then subjected to reverse transcription reactions with (RT+) and without (RT-) the presence of reverse transcriptase. TaqMan assays were carried out with gene-specific primers (see above) and 25 the contribution of genomic DNA to the signal detected was evaluated by comparing the threshold cycles obtained with the RT+/RT- non-Dnase treated RNA to that on the RT+/RT- Dnase treated RNA. The amount of signal contributed by genomic DNA in the Dnased RT- RNA must be less than 10% of that obtained with Dnased RT+ RNA. If not the RNA was not used in actual experiments.

30

35

5 **Reverse Transcription reaction and Sequence Detection**

100ng of Dnase-treated total RNA was annealed to 2.5  $\mu$ M of the respective gene-specific reverse primer in the presence of 5.5 mM Magnesium Chloride by heating the sample to 72°C for 2 min and then cooling to 55° C for 30 min. 1.25 U/ $\mu$ l of MuLv reverse transcriptase and 500 $\mu$ M of each dNTP was added to the reaction and the tube 10 was incubated at 37° C for 30 min. The sample was then heated to 90°C for 5 min to denature enzyme.

Quantitative sequence detection was carried out on an ABI PRISM 7700 by adding to the reverse transcribed reaction 2.5 $\mu$ M forward and reverse primers, 500 $\mu$ M 15 of each dNTP, buffer and 5U AmpliTaq Gold™. The PCR reaction was then held at 94°C for 12 min, followed by 40 cycles of 94° C for 15 sec and 60° C for 30 sec.

**Data handling**

20 The threshold cycle (Ct) of the lowest expressing tissue (the highest Ct value) was used as the baseline of expression and all other tissues were expressed as the relative abundance to that tissue by calculating the difference in Ct value between the baseline and the other tissues and using it as the exponent in  $2^{(\Delta Ct)}$

25 The expanded expression profile of the HGPRBMY2 polypeptide, is provided in Figure 16 and are described elsewhere herein.

**Example 9 - Functional Characterization of the novel human GPCR, HGPRBMY2**

30 The use of mammalian cell reporter assays to demonstrate functional coupling of known GPCRs (G Protein Coupled Receptors) has been well documented in the literature (Gilman, 1987; Boss et al., 1996; Alam & Cook, 1990; George et al., 1997; Selbie & Hill, 1998; Rees et al., 1999). In fact, reporter assays have been successfully used for identifying novel small molecule agonists or antagonists against GPCRs as a class of drug targets (Zlokarnik et al., 1998; George et al., 1997; Boss et al., 1996; Rees et al, 2001).  
35 In such reporter assays, a promoter is regulated as a direct consequence of activation of specific signal transduction cascades following agonist binding to a GPCR (Alam & Cook 1990; Selbie & Hill, 1998; Boss et al., 1996; George et al., 1997; Gilman, 1987).

A number of response element-based reporter systems have been developed that 5 enable the study of GPCR function. These include cAMP response element (CRE)-based reporter genes for G alpha i/o, G alpha s- coupled GPCRs, Nuclear Factor Activator of Transcription (NFAT)-based reporters for G alpha q/11 -coupled receptors and MAP kinase reporter genes for use in Galpha i/o coupled receptors (Selbie & Hill, 1998; Boss et al., 1996; George et al., 1997; Gilman, 1987; Rees et al., 2001). Transcriptional 10 response elements that regulate the expression of Beta-Lactamase within a CHO K1 cell line (Cho/NFAT-CRE: Aurora Biosciences <sup>TM</sup>) (Zlokarnik et al., 1998) have been implemented to characterize the function of the orphan HGPRBMY2 polypeptide of the present invention. The system enables demonstration of constitutive G-protein coupling 15 to endogenous cellular signaling components upon intracellular overexpression of orphan receptors. Overexpression has been shown to represent a physiologically relevant event. For example, it has been shown that overexpression occurs in nature during metastatic carcinomas, wherein defective expression of the monocyte chemotactic protein 1 receptor, CCR2, in macrophages is associated with the incidence of human ovarian 20 carcinoma (Sica, et al., 2000; Salcedo et al., 2000). Indeed, it has been shown that overproduction of the Beta 2 Adrenergic Receptor in transgenic mice leads to constitutive activation of the receptor signaling pathway such that these mice exhibit increased cardiac output (Kypson et al., 1999; Dorn et al., 1999). These are only a few of the many 25 examples demonstrating constitutive activation of GPCRs whereby many of these receptors are likely to be in the active, R\*, conformation (J. Wess 1997).

#### Materials and Methods:

##### 30 DNA Constructs:

The putative GPCR HGPRBMY2 cDNA was PCR amplified using PFU<sup>TM</sup> (Stratagene). The primers used in the PCR reaction were specific to the HGPRBMY2 polynucleotide and were ordered from Gibco BRL (5' prime primer: 5'- 35 CCCAAGCTTATGCAGGCGCTTAACATTACCCCG-3' (SEQ ID NO:17), 3 prime primer: 5'-CGGGATCCTTAATGCCACTGTCTAAAGGAAGA-3' (SEQ ID NO:18). The following 3 prime primer was used to add a Flag-tag epitope to the HGPRBMY2 polypeptide for immunocytochemistry: 5'-

CGGGATCCTTACTTGTCTCGTCGTCCTGTAGTCCATATGCCCACTGTCTAA  
5 AGGAGAATTCTAAC-3' (SEQ ID NO:19). The product from the PCR reaction was isolated from a 0.8% Agarose gel (Invitrogen) and purified using a Gel Extraction Kit <sup>TM</sup> from Qiagen.

10 The purified product was then digested overnight along with the pcDNA3.1 Hygro <sup>TM</sup> mammalian expression vector from Invitrogen using the HindIII and BamHI restriction enzymes (New England Biolabs). These digested products were then purified using the Gel Extraction Kit <sup>TM</sup> from Qiagen and subsequently ligated to the pcDNA3.1 Hygro <sup>TM</sup> expression vector using a DNA molar ratio of 4 parts insert: 1 vector. All DNA modification enzymes were purchased from NEB. The ligation was incubated overnight 15 at 16 degrees Celsius, after which time, one microliter of the mix was used to transform DH5 alpha cloning efficiency competent *E. coli* <sup>TM</sup> (Gibco BRL). A detailed description of the pcDNA3.1 Hygro <sup>TM</sup> mammalian expression vector is available at the Invitrogen web site ([www.Invitrogen.com](http://www.Invitrogen.com)). The plasmid DNA from the ampicillin resistant clones were isolated using the Wizard DNA Miniprep System <sup>TM</sup> from Promega. Positive 20 clones were then confirmed and scaled up for purification using the Qiagen Maxiprep <sup>TM</sup> plasmid DNA purification kit.

#### Cell Line Generation:

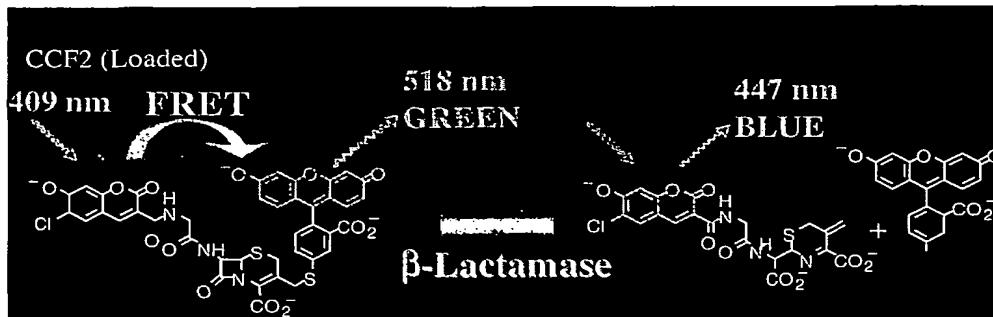
25 The pcDNA3.1hygro vector containing the orphan HGPRBMY2 cDNA were used to transfect Cho/NFAT-CRE (Aurora Biosciences) cells using Lipofectamine 2000 <sup>TM</sup> according to the manufacturers specifications (Gibco BRL). Two days later, the cells were split 1:3 into selective media (DMEM 11056, 600 ug/ml Hygromycin, 200 ug/ml Zeocin, 10% FBS). All cell culture reagents were purchased from Gibco BRL-Invitrogen.

30 The Cho/NFAT-CRE cell lines, transiently or stably transfected with the orphan HGPRBMY2 GPCR, were analyzed using the FACS Vantage SE <sup>TM</sup> (BD), fluorescence microscopy (Nikon), and the L JL Analyst <sup>TM</sup> (Molecular Devices). In this system, changes in real-time gene expression, as a consequence of constitutive G-protein coupling of the orphan HGPRBMY2 GPCR, is examined by analyzing the fluorescence 35 emission of the transformed cells at 447nm and 518nm. The changes in gene expression can be visualized using Beta-Lactamase as a reporter, that, when induced by the appropriate signaling cascade, hydrolyzes an intracellularly loaded, membrane-permeant

ester substrate (CCF2/AM<sup>TM</sup> Aurora Biosciences; Zlokarnik, et al., 1998). The 5 CCF2/AM<sup>TM</sup> substrate is a 7-hydroxycoumarin cephalosporin with a fluorescein attached through a stable thioether linkage. Induced expression of the Beta-Lactamase enzyme is readily apparent since each enzyme molecule produced is capable of changing the fluorescence of many CCF2/AM<sup>TM</sup> substrate molecules. A schematic of this cell based 10 system is shown below.

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20

In summary, CCF2/AM<sup>TM</sup> is a membrane permeant, intracellularly-trapped, 25 fluorescent substrate with a cephalosporin core that links a 7-hydroxycoumarin to a fluorescein. For the intact molecule, excitation of the coumarin at 409 nm results in Fluorescence Resonance Energy Transfer (FRET) to the fluorescein which emits green light at 518 nm. Production of active Beta-Lactamase results in cleavage of the Beta-Lactam ring, leading to disruption of FRET, and excitation of the coumarin only - thus 25 giving rise to blue fluorescent emission at 447 nm.

Fluorescent emissions were detected using a Nikon-TE300 microscope equipped 30 with an excitation filter (D405/10X-25), dichroic reflector (430DCLP), and a barrier filter for dual DAPI/FITC (510nM) to visually capture changes in Beta-Lactamase expression. The FACS Vantage SE is equipped with a Coherent Enterprise II Argon Laser and a Coherent 302C Krypton laser. In flow cytometry, UV excitation at 351-364 nm from the 35 Argon Laser or violet excitation at 407 nm from the Krypton laser are used. The optical filters on the FACS Vantage SE are HQ460/50m and HQ535/40m bandpass separated by a 490 dichroic mirror.

Prior to analyzing the fluorescent emissions from the cell lines as described

above, the cells were loaded with the CCF2/AM substrate. A 6X CCF2/AM loading  
5 buffer was prepared whereby 1mM CCF2/AM (Aurora Biosciences) was dissolved in  
100% DMSO (Sigma). 12 ul of this stock solution was added to 60 ul of 100mg/ml  
Pluronic F127 (Sigma) in DMSO containing 0.1% Acetic Acid (Sigma). This solution  
was added while vortexing to 1 mL of Sort Buffer (PBS minus calcium and magnesium-  
10 Gibco-25 mM HEPES-Gibco- pH 7.4, 0.1% BSA). Cells were placed in serum-free  
media and the 6X CCF2/AM was added to a final concentration of 1X. The cells were  
then loaded at room temperature for one to two hours, and then subjected to fluorescent  
emission analysis as described herein. Additional details relative to the cell loading  
methods and/or instrument settings may be found by reference to the following  
15 publications: see Zlokarnik, et al., 1998; Whitney et al., 1998; and BD Biosciences, 1999.

**Immunocytochemistry:**

The cell lines transfected and selected for expression of Flag-epitope tagged  
orphan GPCRs were analyzed by immunocytochemistry. The cells were plated at  
20 1X10<sup>3</sup> in each well of a glass slide (VWR). The cells were rinsed with PBS followed  
by acid fixation for 30 minutes at room temperature using a mixture of 5% Glacial Acetic  
Acid / 90% ETOH. The cells were then blocked in 2% BSA and 0.1%Triton in PBS,  
incubated for 2 h at room temperature or overnight at 4°C. A monoclonal anti-Flag FITC  
25 antibody was diluted at 1:50 in blocking solution and incubated with the cells for 2 h at  
room temperature. Cells were then washed three times with 0.1%Triton in PBS for five  
minutes. The slides were overlayed with mounting media dropwise with Biomedia -Gel  
Mount<sup>TM</sup> (Biomedia; Containing Anti-Quenching Agent). Cells were examined at 10x  
magnification using the Nikon TE300 equiped with FITC filter (535nm).

30

**Results - HGPRBMY2 constitutively activates gene expression through the NFAT  
response element.**

35 There is strong evidence that certain GPCRs exhibit a cDNA concentration-  
dependent constitutive activity through cAMP response element (CRE) luciferase  
reporters (Chen et al., 1999). In an effort to demonstrate functional coupling of  
HGPRBMY2 to known GPCR second messenger pathways, the HGPRBMY2

polypeptide was expressed at high constitutive levels in the Cho-NFAT/CRE cell line. 5 To this end, the HGPRBMY2 cDNA was PCR amplified and subcloned into the pcDNA3.1 hygro <sup>TM</sup> mammalian expression vector as described herein. Early passage Cho-NFAT/CRE cells were then transfected with the resulting pcDNA3.1 hygro <sup>TM</sup> / HGPRBMY2 construct. Transfected and non-transfected Cho-NFAT/CRE cells (control) were loaded with the CCF2 substrate and stimulated with 10 nM PMA, and 1 uM 10 Thapsigargin (NFAT stimulator) or 10 uM Forskolin (CRE stimulator) to fully activate the NFAT/CRE element. The cells were then analyzed for fluorescent emission by FACS.

The FACS profile demonstrates the constitutive activity of HGPRBMY2 in the Cho-NFAT/CRE line as evidenced by the significant population of cells with blue 15 fluorescent emission at 447 nm (see Figure 12: Blue Cells). As expected, the NFAT/CRE response element in the untransfected control cell line was not activated (i.e., beta lactamase not induced), enabling the CCF2 substrate to remain intact, and resulting in the green fluorescent emission at 518 nM (see Figure 11-Green Cells). A very low level of 20 leaky Beta Lactamase expression was detectable as evidenced by the small population of cells emitting at 447 nm. Analysis of a stable pool of cells transfected with HGPRBMY2 revealed constitutive coupling of the cell population to the NFAT/CRE response element, activation of Beta Lactamase and cleavage of the substrate (Figure 12-Blue Cells). These 25 results demonstrate that overexpression of HGPRBMY2 leads to constitutive coupling of signaling pathways known to be mediated by Gq/11 or Gs coupled receptors that converge to activate either the NFAT or CRE response elements respectively (Boss et al., 1996; Chen et al., 1999).

In an effort to further characterize the observed functional coupling of the HGPRBMY2 polypeptide, its ability to couple to the cAMP response element (CRE) 30 independent of the NFAT response element was examined. To this end, HEK-CRE cell line that contained only the integrated 3XCRE linked to the Beta-Lactamase reporter was transfected with the pcDNA3.1 hygro <sup>TM</sup> / HGPRBMY2 construct. Analysis of the fluorescence emission from this stable pool showed that HGPRBMY2 does not 35 constitutively couple to the cAMP mediated second messenger pathways (see Figure 13). Experiments have shown that known Gs coupled receptors do demonstrate constitutive activation when overexpressed in the HEK-CRE cell line. For example, direct activation of adenylate cyclase using 10 uM Forskolin has been shown to activate CRE and the

subsequent induction of Beta-Lactamase in the HEK-CRE cell line (data not shown). In  
5 conclusion, the results are consistent with HGPRBMY2 representing a functional GPCR  
analogous to known Gq coupled receptors. Therefore, constitutive expression of  
HGPRBMY2 in the CHO Nfat/CRE cell line leads to NFAT activation through  
accumulation of intracellular Ca<sup>2+</sup> as has been demonstrated for the M3 muscarinic  
receptor (Boss et al., 1996).

10 In preferred embodiments, the HGPRBMY2 polynucleotides and polypeptides,  
including agonists, antagonists, and fragments thereof, are useful for modulating  
intracellular Ca<sup>2+</sup> levels, modulating Ca<sup>2+</sup> sensitive signaling pathways, and modulating  
NFAT element associated signaling pathways.

15

Demonstration of Cell Surface Expression:

HGPRBMY2 was tagged at the C-terminus using the Flag epitope and inserted  
into the pcDNA3.1 hygro <sup>TM</sup> expression vector, as described herein.  
Immunocytochemistry of Cho Nfat-CRE cell lines transfected with the Flag-tagged  
20 HGPRBMY2 construct with FITC conjugated Anti Flag monoclonal antibody  
demonstrated that HGPRBMY2 is indeed a cell surface receptor. The  
immunocytochemistry also confirmed expression of the HGPRBMY2 in the Cho Nfat-  
CRE cell lines. Briefly, Cho Nfat-CRE cell lines were transfected with pcDNA3.1 hygro  
25 <sup>TM</sup> / HGPRBMY2-Flag vector, fixed with 70% methanol, and permeabilized with  
0.1%TritonX100. The cells were then blocked with 1% Serum and incubated with a FITC  
conjugated Anti Flag monoclonal antibody at 1:50 dilution in PBS-Triton. The cells were  
then washed several times with PBS-Triton, overlayed with mounting solution, and  
fluorescent images were captured (see Figure 14). The control cell line, non-transfected  
30 ChoNfat CRE cell line, exhibited no detectable background fluorescence (Data not  
shown). The BMY2 -FLAG tagged expressing Cho Nfat CRE line exhibited specific  
plasma membrane expression as indicated (Panel B). These data provide clear evidence  
that BMY2 is expressed at the plasma membrane. Plasma membrane localization in  
35 consistent with HGPRBMY2 representing a 7 transmembrane domain containing GPCR.  
Taken together, the data indicates that HGPRBMY2 is a cell surface GPCR that functions  
through increases in Ca<sup>2+</sup> signal transduction pathways.

Screening Paradigm

5        The Aurora Beta-Lactamase technology provides a clear path for identifying agonists and antagonists of the HGPRBMY2 polypeptide. Cell lines that exhibit a range of constitutive coupling activity have been identified by sorting through HGPRBMY2 transfected cell lines using the FACS Vantage SE (see Figure 15). For example, cell lines have been sorted that have an intermediate level of HGPRBMY2 expression, which also 10 correlates with an intermediate coupling response, using the L JL analyst. Such cell lines will provide the opportunity to screen, indirectly, for both agonists and antagonists of HGPRBMY2 by looking for inhibitors that block the beta lactamase response, or agonists that increase the beta lactamase response. As described herein, modulating the expression 15 level of beta lactamase directly correlates with the level of cleaved CCR2 substrate. For example, this screening paradigm has been shown to work for the identification of modulators of a known GPCR, 5HT6, that couples through Adenylate Cyclase, in addition to, the identification of modulators of the 5HT2c GPCR, that couples through changes in  $[Ca^{2+}]_i$ . The data shown below represent cell lines that have been engineered 20 with the desired pattern of HGPRBMY2 expression to enable the identification of potent small molecule agonists and antagonists. HGPRBMY2 modulator screens may be carried out using a variety of high throughput methods known in the art, though preferably using the fully automated Aurora UHTSS system. The uninduced, HGPRBMY2 transfected 25 Cho Nfat-CRE cell line represents the relative background level of beta lactamase expression (Figure 15; panel a). Following treatment with a cocktail of 10nM Forskolin, 1uM Thapsigargin, and 100 nM PMA (Figure 15; F/T/P; panel b), the cells fully activate the CRE-NFAT response element demonstrating the dynamic range of the assay. Panel C (Figure 15) represents a HGPRBMY2 transfected Cho Nfat-CRE cell line that shows 30 an intermediate level of beta lactamase expression post F/T/P stimulation, while panel D (Figure 15) represents a HGPRBMY2 transfected Cho Nfat-CRE cell line that shows a high level of beta lactamase expression post F/T/P stimulation.

35        In preferred embodiments, the HGPRBMY2 transfected Cho Nfat-CRE cell lines of the present invention are useful for the identification of agonists and antagonists of the HGPRBMY2 polypeptide. Representative uses of these cell lines would be their inclusion in a method of identifying HGPRBMY2 agonists and antagonists. Preferably, the cell lines are useful in a method for identifying a compound that modulates the biological

activity of the HGPRBMY2 polypeptide, comprising the steps of (a) combining a  
5 candidate modulator compound with a host cell expressing the HGPRBMY2 polypeptide  
having the sequence as set forth in SEQ ID NO:14; and (b) measuring an effect of the  
candidate modulator compound on the activity of the expressed HGPRBMY2  
polypeptide. Representative vectors expressing the HGPRBMY2 polypeptide are  
referenced herein (e.g., pcDNA3.1 hygro <sup>TM</sup>) or otherwise known in the art.

10 The cell lines are also useful in a method of screening for a compound that is  
capable of modulating the biological activity of HGPRBMY2 polypeptide, comprising  
the steps of: (a) determining the biological activity of the HGPRBMY2 polypeptide in  
the absence of a modulator compound; (b) contacting a host cell expression the  
15 HGPRBMY2 polypeptide with the modulator compound; and (c) determining the  
biological activity of the HGPRBMY2 polypeptide in the presence of the modulator  
compound; wherein a difference between the activity of the HGPRBMY2 polypeptide  
in the presence of the modulator compound and in the absence of the modulator  
compound indicates a modulating effect of the compound. Additional uses for these cell  
20 lines are described herein or otherwise known in the art

25

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25

30 **Example 10 - Phage Display Methods for Identifying Peptide Ligands or Modulators of Orphan GPCRs**

*Creation of Peptide Libraries.*

Two types of libraries may be created: i.) libraries of 12- and 15 mer peptides for finding peptides that may function as (ant-)agonists and ii.) libraries of peptides with 23-35 33 random residues that are for finding natural ligands through database searches.

The 15 mer library may be i.) an aliquot of the fUSE5-based 15 mer library originally constructed by GP Smith (Scott, JK and Smith, GP. 1990, Science 249, 386-

390). Such a library may be made essentially as described therein, or ii.) a library that is  
5 constructed at Bristol-Myers Squibb in vector M13KE (New England Biolabs) using a  
single-stranded library oligonucleotide extension method (S.S. Sidhu, H.B. Lowman,  
B.C. Cunningham, J.A. Wells: Methods Enzymol., 2000, vol 328, 333-363).

10 The 12 mer library is an aliquot of the M13KE-based 'PhD' 12 mer library (New  
England Biolabs).

10 The libraries with 27-33 random residues are also constructed at Bristol-Myers  
Squibb in vector M13KE (New England Biolabs) using the method described in (S.S.  
Sidhu, H.B. Lowman, B.C. Cunningham, J.A. Wells: Methods Enzymol., 2000, vol 328,  
333-363).

15 All libraries in vector M13KE utilize the standard NNK motif to encode the  
specified number of random residues, where N= A+G+C+T and where K=G+T.

*Sequencing of bound phage:*

20 Standard procedure. Phage in eluates are infected into E. coli host strain (TG1 for  
fUSE5-based 15 mer library; ER2738 (New England Biolabs) for all M13KE-based  
libraries) and are plated for single colonies (fUSE5 vector) or plaques (all M13KE-based  
libraries). Colonies are grown in liquid and sequenced by standard procedure which  
involves 1.) generating PCR product with suitable primers that anneal adjacent to the  
25 library segments in the vectors and 2.) sequencing of the PCR products using one primer  
of each PCR primer pair. Sequences are analyzed for homologies by visual inspection or  
by using the Vector NTI alignment tool.

Peptide Synthesis

30 Peptides are synthesized on Fmoc-Knorr amide resin [N-(9-  
fluorenyl)methoxycarbonyl-Knorr amide-resin, Midwest Biotech, Fishers, Indiana] with  
an Applied Biosystems (Foster City, California) model 433A synthesizer and the *FastMoc*  
chemistry protocol (0.25mmol scale) supplied with the instrument. Amino acids are  
double coupled as their N-alpha-Fmoc- derivatives and reactive side chains are protected  
35 as follows: Asp, Glu: t-Butyl ester (OtBu); Ser, Thr, Tyr: t-Butyl ether (tBu); Asn, Cys,  
Gln, His: Triphenylmethyl (Trt); Lys, Trp: t-Butyloxycarbonyl (Boc); Arg: 2,2,4,6,7-  
Pentamethyldihydrobenzofuran-5-sulfonyl (Pbf). After the final double coupling cycle,

the N-terminal Fmoc group is removed by the multi-step treatment with piperidine in N-  
5 Methylpyrrolidone described by the manufacturer. The N-terminal free amines are then  
treated with 10% acetic anhydride, 5% Diisopropylamine in N-Methylpyrrolidone to yield  
the N-acetyl-derivative. The protected peptidyl-resins are simultaneously deprotected and  
removed from the resin by standard methods. The lyophilized peptides are purified on C<sub>18</sub>  
10 to apparent homogeneity as judged by RP-HPLC analysis. Predicted peptide molecular  
weights are verified by electrospray mass spectrometry.

(J. Biol. Chem.. vol. 273, pp.12041-12046, 1998)

Cyclic analogs are prepared from the crude linear products. The cystine disulfide  
15 may be formed using one of the following methods:

*Method 1:* A sample of the crude peptide is dissolved in water at a concentration of 0.5  
mg/mL and the pH adjusted to 8.5 with NH<sub>4</sub>OH. The reaction is stirred, open to room air,  
and monitored by RP-HPLC.

20 Once complete, the reaction is brought to pH 4 with acetic acid and lyophilized.  
The product is purified and characterized as above.

*Method 2:* A sample of the crude peptide is dissolved at a concentration of 0.5mg/mL in  
25 5% acetic acid. The pH is adjusted to 6.0 with NH<sub>4</sub>OH. DMSO (20% by volume) is  
added and the reaction is stirred overnight. After analytical RP-HPLC analysis, the  
reaction is diluted with H<sub>2</sub>O and triple lyophilized to remove DMSO. The crude product  
is purified by preparative RP-HPLC. (JACS. vol. 113, 6657, 1991)

30 *HGPRBMY2 Peptide Modulators of The Present Invention.*

GDFWYEACESSCAFW (SEQ ID NO:32)

35 LEWGSDVFYDVYDCC (SEQ ID NO:33)

CLRSGTGCAFQLYRF (SEQ ID NO:34)

FAGQIITWYDALDTLM (SEQ ID NO:35)

5

*Assessing Affect of Peptides on GPCR Function.*

The effect of any one of these peptides on the function of the GPCR of the present invention may be determined by adding an effective amount of each peptide to each functional assay. Representative functional assays are described more specifically herein.

10

*Uses of the peptide modulators of the present invention.*

The aforementioned peptides of the present invention are useful for a variety of purposes, though most notably for modulating the function of the GPCR of the present invention, and potentially with other GPCRs of the same G-protein coupled receptor subclass (e.g., peptide receptors, adrenergic receptors, purinergic receptors, etc.), and/or other subclasses known in the art. For example, the peptide modulators of the present invention may be useful as HGPRBMY2 agonists. Alternatively, the peptide modulators of the present invention may be useful as HGPRBMY2 antagonists of the present invention. In addition, the peptide modulators of the present invention may be useful as competitive inhibitors of the HGPRBMY2 cognate ligand(s), or may be useful as non-competitive inhibitors of the HGPRBMY2 cognate ligand(s).

Furthermore, the peptide modulators of the present invention may be useful in assays designed to either deorphan the HGPRBMY2 polypeptide of the present invention, or to identify other agonists or antagonists of the HGPRBMY2 polypeptide of the present invention, particularly small molecule modulators.

**Example 11 – Alternative Method Of Assessing The Ability Of HGPRBMY1 or HGPRBMY2 To Serve As A GPCR Receptor.**

The activity of the HGPRBMY1 or HGPRBMY2 polypeptides may be measured using an assay based upon the property of some known GPCRs to support proliferation in vitro of fibroblasts and tumor cells under serum-free conditions (Chiquet Ehrismann, R. et al. (1986) Cell 47: 131-139). Briefly, wells in 96 well cluster plates (Falcon, Fisher Scientific, Santa Clara CA) are coated with HGPRBMY1 or HGPRBMY2 polypeptides by incubation with solutions at 50-100  $\mu$ g/ml for 15 min at ambient temperature. The coating solution is aspirated, and the wells washed with Dulbecco's medium before cells

are plated. Rat fibroblast cultures or rat mammary tumor cells are prepared as described 5 and plated at a density of 104-105 cells/ml in Dulbecco's medium supplemented with 10% fetal calf serum (FCS).

After three days the media are removed, and the cells washed three times with phosphatebuffered saline (PBS) before the addition of serum-free Dulbecco's medium 10 containing 0.25 mg/ml bovine serum albumin (BSA, Fraction V, Sigma Chemical, St. Louis, MO). After 2 days the medium is aspirated, and 100  $\mu$ l of [<sup>3</sup>H] thymidine (NEN) 15 at 2  $\mu$ Ci/ml in fresh Dulbecco's medium containing 0.25 mg/ml BSA added. Parallel plates are fixed and stained to determine cell numbers. After 16 hr, the medium is aspirated, the cell layer washed with PBS, and the 10% trichloroacetic acid-precipitable counts in the cell layer determined by liquid scintillation counting of radioisotope (normalized to relative cell numbers; Chiquet-Ehrismann, R. et al. (1986) *supra*). The rates of cell proliferation and [<sup>3</sup>H] thymidine uptake are proportional to the levels of GCRP in the sample.

Alternatively, the assay for HGPRBMY1 or HGPRBMY2 polypeptide activity 20 is based upon the property of CD97/Emrl GPCR family proteins to modulate G protein-activated second messenger signal transduction pathways (e. g., cAMP; Gaudin, P. et al. (1998) *J. Biol. Chem.*, 273: 4990-4996). A plasmid encoding the full length HGPRBMY1 or HGPRBMY2 polypeptide is transfected into a mammalian cell line (e. g., COS-7 or 25 Chinese hamster ovary (CHO-K1) cell lines) using methods well-known in the art. Transfected cells are grown in 12-well trays in culture medium containing 2% FCS for 48 hours, the culture medium is discarded, then the attached cells are gently washed with PBS. The cells are then incubated in culture medium with 10% FCS or 2% FCS for 30 minutes, then the medium is removed and cells lysed by treatment with 1 M perchloric 30 acid. The cAMP levels in the lysate are measured by radioimmunoassay using methods well-known in the art. Changes in the levels of cAMP in the lysate from 10% FCS-treated cells compared with those in 2% FCS-treated cells are proportional to the amount of the HGPRBMY1 or HGPRBMY2 polypeptide present in the transfected cells.

35 **Example 12 – Method Of Assessing The Physiological Function Of The HGPRBMY1 or HGPRBMY2 Polypeptide At The Cellular Level.**

The physiological function of the HGPRBMY1 or HGPRBMY2 polypeptide may

be assessed by expressing the sequences encoding HGPRBMY1 or HGPRBMY2 at 5 physiologically elevated levels in mammalian cell culture systems. cDNA is subcloned into a mammalian expression vector containing a strong promoter that drives high levels of cDNA expression (examples are provided elsewhere herein). Vectors of choice include pCMV SPORT (Life Technologies) and pCR3.1 (Invitrogen, Carlsbad CA), both of 10 which contain the cytomegalovirus promoter. 5-10, ug of recombinant vector are transiently transfected into a human cell line, preferably of endothelial or hematopoietic origin, using either liposome formulations or electroporation. 1-2ug of an additional plasmid containing sequences encoding a marker protein are cotransfected. Expression 15 of a marker protein provides a means to distinguish transfected cells from nontransfected cells and is a reliable predictor of cDNA expression from the recombinant vector. Marker proteins of choice include, e.g., Green Fluorescent Protein (GFP; Clontech), CD64, or a CD64-GFP fusion protein. Flow cytometry (FCM), an automated, laser optics-based 20 technique, is used to identify transfected cells expressing GFP or CD64-GFP and to evaluate the apoptotic state of the cells and other cellular properties. FCM detects and quantifies the uptake of fluorescent molecules that diagnose events preceding or 25 coincident with cell death. These events include changes in nuclear DNA content as measured by staining of DNA with propidium iodide; changes in cell size and granularity as measured by forward light scatter and 90 degree side light scatter; down-regulation of DNA synthesis as measured by decrease in bromodeoxyuridine uptake; alterations in 30 expression of cell surface and intracellular proteins as measured by reactivity with specific antibodies; and alterations in plasma membrane composition as measured by the binding of fluorescein-conjugated Annexin V protein to the cell surface. Methods in flow cytometry are discussed in Ormerod, M. G. (1994) *Flow Cytometry*, Oxford, New York NY.

The influence of HGPRBMY1 or HGPRBMY2 polypeptides on gene expression can be assessed using highly purified populations of cells transfected with sequences 35 encoding HGPRBMY1 or HGPRBMY2 and either CD64 or CD64-GFP. CD64 and CD64-GFP are expressed on the surface of transfected cells and bind to conserved regions of human immunoglobulin G (IgG). Transfected cells are efficiently separated from nontransfected cells using magnetic beads coated with either human IgG or antibody against CD64 (DYNAL, Lake Success NY). mRNA can be purified from the cells using

methods well known by those of skill in the art. Expression of mRNA encoding HGPRBMY1 or HGPRBMY2 polypeptides and other genes of interest can be analyzed by northern analysis or microarray techniques.

**Example 13 - Method Of Assessing The Physiological Function Of The HGPRBMY1 or HGPRBMY2 Polypeptides In Xenopus Oocytes.**

10 Capped RNA transcripts from linearized plasmid templates encoding the receptor cDNAs of the invention are synthesized in vitro with RNA polymerases in accordance with standard procedures.

15 In vitro transcripts are suspended in water at a final concentration of 0.2 mg/ml. Ovarian lobes are removed from adult female toads, Stage V defolliculated oocytes are obtained, and RNA transcripts (10 ng/oocyte) are injected in a 50 nl bolus using a microinjection apparatus. Two electrode voltage clamps are used to measure the currents from individual *Xenopus* oocytes in response to agonist exposure. Recordings are made in Ca<sup>2+</sup> free Barth's medium at room temperature.

20 In a preferred embodiment, such a system can be used to screen known ligands and tissue/cell extracts for activating ligands. A number of GPCR ligands are known in the art and are encompassed by the present invention (see, for example, The G-Protein Linked Receptor Facts Book, referenced elsewhere herein).

25 **Example 14 - Method Of Assessing The Physiological Function Of The HGPRBMY1 or HGPRBMY2 Polypeptides Using Microphysiometric Assays.**

Activation of a wide variety of secondary messenger systems results in extrusion of small amounts of acid from a cell. The acid formed is largely as a result of the 30 increased metabolic activity required to fuel the intracellular signaling process. The pH changes in the media surrounding the cell are very small but are detectable by the CYTOSENSOR microphysiometer (Molecular Devices Ltd., Menlo Park, CA). The CYTOSENSOR is thus capable of detecting the activation of a receptor that is coupled to an energy utilizing intracellular signaling pathway such as the G-protein coupled 35 receptor of the present invention.

**Example 15 - Method Of Assessing The Physiological Function Of The**

**HGPRBMY1 or HGPRBMY2 Polypeptides Using Calcium And Camp Functional  
5 Assays.**

A well known observation in the art relates to the fact that GPCR receptors which are expressed in HEK 293 cells have been shown to be functionally couple – leading to subsequent activation of phospholipase C (PLC) and calcium mobilization, and/or cAMP stimulation or inhibition.

10 Based upon the above, calcium and cAMP assays may be useful in assessing the ability of HGPRBMY1 or HGPRBMY2 to serve as a GPCR. Briefly, basal calcium levels in the HEK 293 cells in HGPRBMY1 or HGPRBMY2-transfected or vector control cells can be observed to determine whether the levels fall within a normal physiological range,

15 100 nM to 200 nM. HEK 293 cells expressing recombinant receptors are then loaded with fura 2 and in a single day selected GPCR ligands or tissue/cell extracts are evaluated for agonist induced calcium mobilization. Similarly, HEK 293 cells expressing recombinant HGPRBMY1 or HGPRBMY2 receptors are evaluated for the stimulation or inhibition of cAMP production using standard cAMP quantitation assays. Agonists

20 presenting a calcium transient or cAMP fluctuation are tested in vector control cells to determine if the response is unique to the transfected cells expressing the HGPRBMY1 or HGPRBMY2 receptor.

**25 Example 16 – Method Of Screening For Compounds That Interact With The HGPRBMY1 or HGPRBMY2 Polypeptide.**

The following assays are designed to identify compounds that bind to the HGPRBMY1 or HGPRBMY2 polypeptide, bind to other cellular proteins that interact with the HGPRBMY1 or HGPRBMY2 polypeptide, and to compounds that interfere with 30 the interaction of the HGPRBMY1 or HGPRBMY2 polypeptide with other cellular proteins.

Such compounds can include, but are not limited to, other cellular proteins. Specifically, such compounds can include, but are not limited to, peptides, such as, for 35 example, soluble peptides, including, but not limited to Ig-tailed fusion peptides, comprising extracellular portions of HGPRBMY1 or HGPRBMY2 polypeptide transmembrane receptors, and members of random peptide libraries (see, e.g., Lam, K. S. et al., 1991, *Nature* 354:82-84; Houghton, R. et al., 1991, *Nature* 354:84-86), made of

5 D-and/or L-configuration amino acids, phosphopeptides (including, but not limited to, members of random or partially degenerate phosphopeptide libraries; see, e.g., Songyang, Z., et al., 1993, *Cell* 72:767-778), antibodies (including, but not limited to, polyclonal, monoclonal, humanized, anti-idiotypic, chimeric or single chain antibodies, and FAb, F(ab')<sub>2</sub> and FAb expression library fragments, and epitope-binding fragments thereof), and small organic or inorganic molecules.

10 Compounds identified via assays such as those described herein can be useful, for example, in elaborating the biological function of the HGPRBMY1 or HGPRBMY2 polypeptide, and for ameliorating symptoms of tumor progression, for example. In instances, for example, whereby a tumor progression state or disorder results from a 15 lower overall level of HGPRBMY1 or HGPRBMY2 expression, HGPRBMY1 or HGPRBMY2 polypeptide, and/or HGPRBMY1 or HGPRBMY2 polypeptide activity in a cell involved in the tumor progression state or disorder, compounds that interact with the HGPRBMY1 or HGPRBMY2 polypeptide can include ones which accentuate or amplify the activity of the bound HGPRBMY1 or HGPRBMY2 polypeptide. Such 20 compounds would bring about an effective increase in the level of HGPRBMY1 or HGPRBMY2 polypeptide activity, thus ameliorating symptoms of the tumor progression disorder or state. In instances whereby mutations within the HGPRBMY1 or HGPRBMY2 polypeptide cause aberrant HGPRBMY1 or HGPRBMY2 polypeptides to 25 be made which have a deleterious effect that leads to tumor progression, compounds that bind HGPRBMY1 or HGPRBMY2 polypeptide can be identified that inhibit the activity of the bound HGPRBMY1 or HGPRBMY2 polypeptide. Assays for testing the effectiveness of such compounds are known in the art and discussed, elsewhere herein.

30 **Example 17 – Method Of Screening, In Vitro, Compounds That Bind To The HGPRBMY1 or HGPRBMY2 Polypeptide.**

35 In vitro systems can be designed to identify compounds capable of binding the HGPRBMY1 or HGPRBMY2 polypeptide of the invention. Compounds identified can be useful, for example, in modulating the activity of wild type and/or mutant HGPRBMY1 or HGPRBMY2 polypeptide, preferably mutant HGPRBMY1 or HGPRBMY2 polypeptide, can be useful in elaborating the biological function of the HGPRBMY1 or HGPRBMY2 polypeptide, can be utilized in screens for identifying

compounds that disrupt normal HGPRBMY1 or HGPRBMY2 polypeptide interactions,  
5 or can in themselves disrupt such interactions.

The principle of the assays used to identify compounds that bind to the HGPRBMY1 or HGPRBMY2 polypeptide involves preparing a reaction mixture of the HGPRBMY1 or HGPRBMY2 polypeptide and the test compound under conditions and for a time sufficient to allow the two components to interact and bind, thus forming a  
10 complex which can be removed and/or detected in the reaction mixture. These assays can be conducted in a variety of ways. For example, one method to conduct such an assay would involve anchoring HGPRBMY1 or HGPRBMY2 polypeptide or the test substance onto a solid phase and detecting HGPRBMY1 or HGPRBMY2 polypeptide /test  
15 compound complexes anchored on the solid phase at the end of the reaction. In one embodiment of such a method, the HGPRBMY1 or HGPRBMY2 polypeptide can be anchored onto a solid surface, and the test compound, which is not anchored, can be labeled, either directly or indirectly.

In practice, microtitre plates can conveniently be utilized as the solid phase. The  
20 anchored component can be immobilized by non-covalent or covalent attachments. Non-covalent attachment can be accomplished by simply coating the solid surface with a solution of the protein and drying. Alternatively, an immobilized antibody, preferably a monoclonal antibody, specific for the protein to be immobilized can be used to anchor  
25 the protein to the solid surface. The surfaces can be prepared in advance and stored.

In order to conduct the assay, the nonimmobilized component is added to the coated surface containing the anchored component. After the reaction is complete, unreacted components are removed (e.g., by washing) under conditions such that any complexes formed will remain immobilized on the solid surface. The detection of  
30 complexes anchored on the solid surface can be accomplished in a number of ways. Where the previously immobilized component is pre-labeled, the detection of label immobilized on the surface indicates that complexes were formed. Where the previously nonimmobilized component is not pre-labeled, an indirect label can be used to detect  
35 complexes anchored on the surface; e.g., using a labeled antibody specific for the immobilized component (the antibody, in turn, can be directly labeled or indirectly labeled with a labeled anti-Ig antibody).

Alternatively, a reaction can be conducted in a liquid phase, the reaction products

separated from unreacted components, and complexes detected; e.g., using an  
5 immobilized antibody specific for HGPRBMY1 or HGPRBMY2 polypeptide or the test  
compound to anchor any complexes formed in solution, and a labeled antibody specific  
for the other component of the possible complex to detect anchored complexes.

10 **Example 18 – Method For Identifying A Putative Ligand For The HGCRBMY1 or  
HGPRBMY2 Polypeptide.**

Ligand binding assays provide a direct method for ascertaining receptor pharmacology and are adaptable to a high throughput format. A panel of known GPCR purified ligands may be radiolabeled to high specific activity (50-2000 Ci/mmol) for  
15 binding studies. A determination is then made that the process of radiolabeling does not diminish the activity of the ligand towards its receptor. Assay conditions for buffers, ions, pH and other modulators such as nucleotides are optimized to establish a workable signal to noise ratio for both membrane and whole cell receptor sources. For these assays, specific receptor binding is defined as total associated radioactivity minus the  
20 radioactivity measured in the presence of an excess of unlabeled competing ligand. Where possible, more than one competing ligand is used to define residual nonspecific binding.

A number of GPCR ligands are known in the art and are encompassed by the  
25 present invention (see, for example, The G-Protein Linked Receptor Facts Book, referenced elsewhere herein).

Alternatively, the HGPRBMY1 or HGPRBMY2 polypeptide of the present invention may also be functionally screened (using calcium, cAMP, microphysiometer, oocyte electrophysiology, etc., functional screens) against tissue extracts to identify  
30 natural ligands. Extracts that produce positive functional responses can be sequentially subfractionated until an activating ligand is isolated identified using methods well known in the art, some of which are described herein.

35 **Example 19 – Method Of Identifying Compounds That Interfere With HGPRBMY1 or HGPRBMY2 Polypeptide/Cellular Product Interaction.**

The HGPRBMY1 or HGPRBMY2 polypeptide of the invention can, *in vivo*,

interact with one or more cellular or extracellular macromolecules, such as proteins. Such 5 macromolecules include, but are not limited to, polypeptides, particularly GPCR ligands, and those products identified via screening methods described, elsewhere herein. For the purposes of this discussion, such cellular and extracellular macromolecules are referred to herein as "binding partner(s)". For the purpose of the present invention, "binding 10 partner" may also encompass polypeptides, small molecule compounds, polysaccharides, lipids, and any other molecule or molecule type referenced herein. Compounds that disrupt such interactions can be useful in regulating the activity of the HGPRBMY1 or HGPRBMY2 polypeptide, especially mutant HGPRBMY1 or HGPRBMY2 polypeptide. Such compounds can include, but are not limited to molecules such as antibodies, 15 peptides, and the like described in elsewhere herein.

The basic principle of the assay systems used to identify compounds that interfere with the interaction between the HGPRBMY1 or HGPRBMY2 polypeptide and its cellular or extracellular binding partner or partners involves preparing a reaction mixture containing the HGPRBMY1 or HGPRBMY2 polypeptide, and the binding partner under 20 conditions and for a time sufficient to allow the two products to interact and bind, thus forming a complex. In order to test a compound for inhibitory activity, the reaction mixture is prepared in the presence and absence of the test compound. The test compound can be initially included in the reaction mixture, or can be added at a time subsequent to 25 the addition of HGPRBMY1 or HGPRBMY2 polypeptide and its cellular or extracellular binding partner. Control reaction mixtures are incubated without the test compound or with a placebo. The formation of any complexes between the HGPRBMY1 or HGPRBMY2 polypeptide and the cellular or extracellular binding partner is then detected. The formation of a complex in the control reaction, but not in the reaction 30 mixture containing the test compound, indicates that the compound interferes with the interaction of the HGPRBMY1 or HGPRBMY2 polypeptide and the interactive binding partner. Additionally, complex formation within reaction mixtures containing the test compound and normal HGPRBMY1 or HGPRBMY2 polypeptide can also be compared to complex formation within reaction mixtures containing the test compound and mutant 35 HGPRBMY1 or HGPRBMY2 polypeptide. This comparison can be important in those cases wherein it is desirable to identify compounds that disrupt interactions of mutant but not normal HGPRBMY1 or HGPRBMY2 polypeptide.

The assay for compounds that interfere with the interaction of the HGPRBMY1 or HGPRBMY2 polypeptide and binding partners can be conducted in a heterogeneous or homogeneous format. Heterogeneous assays involve anchoring either the HGPRBMY1 or HGPRBMY2 polypeptide or the binding partner onto a solid phase and detecting complexes anchored on the solid phase at the end of the reaction. In homogeneous assays, the entire reaction is carried out in a liquid phase. In either approach, the order of addition of reactants can be varied to obtain different information about the compounds being tested. For example, test compounds that interfere with the interaction between the HGPRBMY1 or HGPRBMY2 polypeptide and the binding partners, e.g., by competition, can be identified by conducting the reaction in the presence of the test substance; i.e., by adding the test substance to the reaction mixture prior to or simultaneously with the HGPRBMY1 or HGPRBMY2 polypeptide and interactive cellular or extracellular binding partner. Alternatively, test compounds that disrupt preformed complexes, e.g. compounds with higher binding constants that displace one of the components from the complex, can be tested by adding the test compound to the reaction mixture after complexes have been formed. The various formats are described briefly below.

In a heterogeneous assay system, either the HGPRBMY1 or HGPRBMY2 polypeptide or the interactive cellular or extracellular binding partner, is anchored onto a solid surface, while the non-anchored species is labeled, either directly or indirectly. In practice, microtitre plates are conveniently utilized. The anchored species can be immobilized by non-covalent or covalent attachments. Non-covalent attachment can be accomplished simply by coating the solid surface with a solution of the HGPRBMY1 or HGPRBMY2 polypeptide or binding partner and drying. Alternatively, an immobilized antibody specific for the species to be anchored can be used to anchor the species to the solid surface. The surfaces can be prepared in advance and stored.

In order to conduct the assay, the partner of the immobilized species is exposed to the coated surface with or without the test compound. After the reaction is complete, unreacted components are removed (e.g., by washing) and any complexes formed will remain immobilized on the solid surface. The detection of complexes anchored on the solid surface can be accomplished in a number of ways. Where the non-immobilized species is pre-labeled, the detection of label immobilized on the surface indicates that complexes were formed. Where the non-immobilized species is not pre-labeled, an

5 indirect label can be used to detect complexes anchored on the surface; e.g., using a labeled antibody specific for the initially non-immobilized species (the antibody, in turn, can be directly labeled or indirectly labeled with a labeled anti-Ig antibody). Depending upon the order of addition of reaction components, test compounds which inhibit complex formation or which disrupt preformed complexes can be detected.

10 Alternatively, the reaction can be conducted in a liquid phase in the presence or absence of the test compound, the reaction products separated from unreacted components, and complexes detected; e.g., using an immobilized antibody specific for one of the binding components to anchor any complexes formed in solution, and a labeled antibody specific for the other partner to detect anchored complexes. Again, depending 15 upon the order of addition of reactants to the liquid phase, test compounds which inhibit complex or which disrupt preformed complexes can be identified.

20 In an alternate embodiment of the invention, a homogeneous assay can be used. In this approach, a preformed complex of the HGPRBMY1 or HGPRBMY2 polypeptide and the interactive cellular or extracellular binding partner product is prepared in which either the HGPRBMY1 or HGPRBMY2 polypeptide or their binding partners are labeled, but the signal generated by the label is quenched due to complex formation (see, e.g., U.S. Pat. No. 4,109,496 by Rubenstein which utilizes this approach for immunoassays).

25 The addition of a test substance that competes with and displaces one of the species from the preformed complex will result in the generation of a signal above background. In this way, test substances which disrupt HGPRBMY1 or HGPRBMY2 polypeptide -cellular or extracellular binding partner interaction can be identified.

30 In a particular embodiment, the HGPRBMY1 or HGPRBMY2 polypeptide can be prepared for immobilization using recombinant DNA techniques known in the art. For example, the HGPRBMY1 or HGPRBMY2 polypeptide coding region can be fused to a glutathione-S-transferase (GST) gene using a fusion vector such as pGEX-5X-1, in such a manner that its binding activity is maintained in the resulting fusion product. The interactive cellular or extracellular product can be purified and used to raise a monoclonal antibody, using methods routinely practiced in the art and described above. This antibody 35 can be labeled with the radioactive isotope <sup>125</sup>I, for example, by methods routinely practiced in the art. In a heterogeneous assay, e.g., the GST- HGPRBMY1 or

HGPRBMY2 polypeptide fusion product can be anchored to glutathione-agarose beads.

5     The interactive cellular or extracellular binding partner product can then be added in the presence or absence of the test compound in a manner that allows interaction and binding to occur. At the end of the reaction period, unbound material can be washed away, and the labeled monoclonal antibody can be added to the system and allowed to bind to the 10 complexed components. The interaction between the HGPRBMY1 or HGPRBMY2 polypeptide and the interactive cellular or extracellular binding partner can be detected by measuring the amount of radioactivity that remains associated with the glutathione-agarose beads. A successful inhibition of the interaction by the test compound will result in a decrease in measured radioactivity.

15     Alternatively, the GST- HGPRBMY1 or HGPRBMY2 polypeptide fusion product and the interactive cellular or extracellular binding partner product can be mixed together in liquid in the absence of the solid glutathione-agarose beads. The test compound can be added either during or after the binding partners are allowed to interact. This mixture can then be added to the glutathione-agarose beads and unbound material is washed 20 away. Again the extent of inhibition of the binding partner interaction can be detected by adding the labeled antibody and measuring the radioactivity associated with the beads.

25     In another embodiment of the invention, these same techniques can be employed using peptide fragments that correspond to the binding domains of the HGPRBMY1 or HGPRBMY2 polypeptide product and the interactive cellular or extracellular binding partner (in case where the binding partner is a product), in place of one or both of the full length products.

30     Any number of methods routinely practiced in the art can be used to identify and isolate the protein's binding site. These methods include, but are not limited to, mutagenesis of one of the genes encoding one of the products and screening for disruption of binding in a co-immunoprecipitation assay. Compensating mutations in the gene encoding the second species in the complex can be selected. Sequence analysis of the genes encoding the respective products will reveal the mutations that correspond to the region of the product involved in interactive binding. Alternatively, one product can 35 be anchored to a solid surface using methods described in this Section above, and allowed to interact with and bind to its labeled binding partner, which has been treated with a proteolytic enzyme, such as trypsin. After washing, a short, labeled peptide

comprising the binding domain can remain associated with the solid material, which can  
5 be isolated and identified by amino acid sequencing. Also, once the gene coding for the  
cellular or extracellular binding partner product is obtained, short gene segments can be  
engineered to express peptide fragments of the product, which can then be tested for  
binding activity and purified or synthesized.

10

**Example 20 - Isolation Of A Specific Clone From The Deposited Sample.**

The deposited material in the sample assigned the ATCC Deposit Number cited  
herein for any given cDNA clone also may contain one or more additional plasmids, each  
comprising a cDNA clone different from that given clone. Thus, deposits sharing the  
15 same ATCC Deposit Number contain at least a plasmid for each cDNA clone identified  
herein. Typically, each ATCC deposit sample cited herein comprises a mixture of  
approximately equal amounts (by weight) of about 1-10 plasmid DNAs, each containing  
a different cDNA clone and/or partial cDNA clone; but such a deposit sample may  
include plasmids for more or less than 2 cDNA clones.

20

Two approaches can be used to isolate a particular clone from the deposited  
sample of plasmid DNA(s) of the present invention. First, a plasmid is directly isolated  
by screening the clones using a polynucleotide probe corresponding to SEQ ID NO:1 or  
SEQ ID NO:13.

25

Particularly, a specific polynucleotide with 30-40 nucleotides is synthesized using  
an Applied Biosystems DNA synthesizer according to the sequence reported. The  
oligonucleotide is labeled, for instance, with 32P-( $\gamma$ -ATP using T4 polynucleotide kinase  
and purified according to routine methods. (E.g., Maniatis et al., Molecular Cloning: A  
Laboratory Manual, Cold Spring Harbor Press, Cold Spring, NY (1982).) The plasmid  
30 mixture is transformed into a suitable host, as indicated above (such as XL-1 Blue  
(Stratagene)) using techniques known to those of skill in the art, such as those provided  
by the vector supplier or in related publications or patents cited above. The transformants  
are plated on 1.5% agar plates (containing the appropriate selection agent, e.g.,  
35 ampicillin) to a density of about 150 transformants (colonies) per plate. These plates are  
screened using Nylon membranes according to routine methods for bacterial colony  
screening (e.g., Sambrook et al., Molecular Cloning: A Laboratory Manual, 2nd Edit.,

(1989), Cold Spring Harbor Laboratory Press, pages 1.93 to 1.104), or other techniques  
5 known to those of skill in the art.

Alternatively, two primers of 17-20 nucleotides derived from both ends of the SEQ ID NO:1 or SEQ ID NO:13 (i.e., within the region of SEQ ID NO:1 or SEQ ID NO:13 bounded by the 5' NT and the 3' NT of the clone defined herein) are synthesized and used to amplify the desired cDNA using the deposited cDNA plasmid as a template.  
10 The polymerase chain reaction is carried out under routine conditions, for instance, in 25  $\mu$ l of reaction mixture with 0.5 ug of the above cDNA template. A convenient reaction mixture is 1.5-5 mM MgCl<sub>2</sub>, 0.01% (w/v) gelatin, 20  $\mu$ M each of dATP, dCTP, dGTP, dTTP, 25 pmol of each primer and 0.25 Unit of Taq polymerase. Thirty five cycles of  
15 PCR (denaturation at 94 degree C for 1 min; annealing at 55 degree C for 1 min; elongation at 72 degree C for 1 min) are performed with a Perkin-Elmer Cetus automated thermal cycler. The amplified product is analyzed by agarose gel electrophoresis and the DNA band with expected molecular weight is excised and purified. The PCR product is verified to be the selected sequence by subcloning and sequencing the DNA product.

20 The polynucleotide(s) of the present invention, the polynucleotide encoding the polypeptide of the present invention, or the polypeptide encoded by the deposited clone may represent partial, or incomplete versions of the complete coding region (i.e., full-length gene). Several methods are known in the art for the identification of the 5' or 3' 25 non-coding and/or coding portions of a gene which may not be present in the deposited clone. The methods that follow are exemplary and should not be construed as limiting the scope of the invention. These methods include but are not limited to, filter probing, clone enrichment using specific probes, and protocols similar or identical to 5' and 3' "RACE" protocols that are well known in the art. For instance, a method similar to 5' RACE is 30 available for generating the missing 5' end of a desired full-length transcript. (Fromont-Racine et al., Nucleic Acids Res. 21(7):1683-1684 (1993)).

35 Briefly, a specific RNA oligonucleotide is ligated to the 5' ends of a population of RNA presumably containing full-length gene RNA transcripts. A primer set containing a primer specific to the ligated RNA oligonucleotide and a primer specific to a known sequence of the gene of interest is used to PCR amplify the 5' portion of the desired full-length gene. This amplified product may then be sequenced and used to generate the full-length gene.

This above method starts with total RNA isolated from the desired source, 5 although poly-A+ RNA can be used. The RNA preparation can then be treated with phosphatase if necessary to eliminate 5' phosphate groups on degraded or damaged RNA that may interfere with the later RNA ligase step. The phosphatase should then be inactivated and the RNA treated with tobacco acid pyrophosphatase in order to remove the cap structure present at the 5' ends of messenger RNAs. This reaction leaves a 5' 10 phosphate group at the 5' end of the cap cleaved RNA which can then be ligated to an RNA oligonucleotide using T4 RNA ligase.

This modified RNA preparation is used as a template for first strand cDNA synthesis using a gene specific oligonucleotide. The first strand synthesis reaction is used 15 as a template for PCR amplification of the desired 5' end using a primer specific to the ligated RNA oligonucleotide and a primer specific to the known sequence of the gene of interest. The resultant product is then sequenced and analyzed to confirm that the 5' end sequence belongs to the desired gene. Moreover, it may be advantageous to optimize the 20 RACE protocol to increase the probability of isolating additional 5' or 3' coding or non-coding sequences. Various methods of optimizing a RACE protocol are known in the art, though a detailed description summarizing these methods can be found in B.C. Schaefer, Anal. Biochem., 227:255-273, (1995).

An alternative method for carrying out 5' or 3' RACE for the identification of 25 coding or non-coding sequences is provided by Frohman, M.A., et al., Proc.Nat'l.Acad.Sci.USA, 85:8998-9002 (1988). Briefly, a cDNA clone missing either the 5' or 3' end can be reconstructed to include the absent base pairs extending to the translational start or stop codon, respectively. In some cases, cDNAs are missing the start 30 of translation, therefor. The following briefly describes a modification of this original 5' RACE procedure. Poly A+ or total RNAs reverse transcribed with Superscript II (Gibco/BRL) and an antisense or I complementary primer specific to the cDNA sequence. The primer is removed from the reaction with a Microcon Concentrator (Amicon). The first-strand cDNA is then tailed with dATP and terminal deoxynucleotide transferase 35 (Gibco/BRL). Thus, an anchor sequence is produced which is needed for PCR amplification. The second strand is synthesized from the dA-tail in PCR buffer, Taq DNA polymerase (Perkin-Elmer Cetus), an oligo-dT primer containing three adjacent restriction sites (XhoI/Sail and ClaI) at the 5' end and a primer containing just these

restriction sites. This double-stranded cDNA is PCR amplified for 40 cycles with the  
5 same primers as well as a nested cDNA-specific antisense primer. The PCR products are  
size-separated on an ethidium bromide-agarose gel and the region of gel containing  
cDNA products the predicted size of missing protein-coding DNA is removed. cDNA is  
purified from the agarose with the Magic PCR Prep kit (Promega), restriction digested  
10 with XhoI or SalI, and ligated to a plasmid such as pBluescript SKII (Stratagene) at XhoI  
and EcoRV sites. This DNA is transformed into bacteria and the plasmid clones  
sequenced to identify the correct protein-coding inserts. Correct 5' ends are confirmed by  
comparing this sequence with the putatively identified homologue and overlap with the  
partial cDNA clone. Similar methods known in the art and/or commercial kits are used  
15 to amplify and recover 3' ends.

Several quality-controlled kits are commercially available for purchase. Similar  
reagents and methods to those above are supplied in kit form from Gibco/BRL for both  
5' and 3'RACE for recovery of full length genes. A second kit is available from Clontech  
which is a modification of a related technique, SLIC (single-stranded ligation to single-  
20 stranded cDNA), developed by Dumas et al., Nucleic Acids Res., 19:5227-32(1991). The  
major differences in procedure are that the RNA is alkaline hydrolyzed after reverse  
transcription and RNA ligase is used to join a restriction site-containing anchor primer  
to the first-strand cDNA. This obviates the necessity for the dA-tailing reaction which  
25 results in a polyT stretch that is difficult to sequence past.

An alternative to generating 5' or 3' cDNA from RNA is to use cDNA library  
double-stranded DNA. An asymmetric PCR-amplified antisense cDNA strand is  
synthesized with an antisense cDNA-specific primer and a plasmid-anchored primer.  
These primers are removed and a symmetric PCR reaction is performed with a nested  
30 cDNA-specific antisense primer and the plasmid-anchored primer.

*RNA Ligase Protocol For Generating The 5' or 3' End Sequences To Obtain Full Length  
Genes*

Once a gene of interest is identified, several methods are available for the  
35 identification of the 5' or 3' portions of the gene which may not be present in the original  
cDNA plasmid. These methods include, but are not limited to, filter probing, clone  
enrichment using specific probes and protocols similar and identical to 5' and 3'RACE.

While the full-length gene may be present in the library and can be identified by probing, 5 a useful method for generating the 5' or 3' end is to use the existing sequence information from the original cDNA to generate the missing information. A method similar to 5'RACE is available for generating the missing 5' end of a desired full-length gene. (This method was published by Fromont-Racine et al., Nucleic Acids Res., 21(7): 1683-1684 10 (1993)). Briefly, a specific RNA oligonucleotide is ligated to the 5' ends of a population of RNA presumably 30 containing full-length gene RNA transcript and a primer set containing a primer specific to the ligated RNA oligonucleotide and a primer specific to a known sequence of the gene of interest, is used to PCR amplify the 5' portion of the desired full length gene which may then be sequenced and used to generate the full length 15 gene. This method starts with total RNA isolated from the desired source, poly A RNA may be used but is not a prerequisite for this procedure. The RNA preparation may then be treated with phosphatase if necessary to eliminate 5' phosphate groups on degraded or damaged RNA which may interfere with the later RNA ligase step. The phosphatase if used is then inactivated and the RNA is treated with tobacco acid pyrophosphatase in 20 order to remove the cap structure present at the 5' ends of messenger RNAs. This reaction leaves a 5' phosphate group at the 5' end of the cap cleaved RNA which can then be ligated to an RNA oligonucleotide using T4 RNA ligase. This modified RNA preparation can then be used as a template for first strand cDNA synthesis using a gene specific 25 oligonucleotide. The first strand synthesis reaction can then be used as a template for PCR amplification of the desired 5' end using a primer specific to the ligated RNA oligonucleotide and a primer specific to the known sequence of the apoptosis related of interest. The resultant product is then sequenced and analyzed to confirm that the 5' end sequence belongs to the relevant apoptosis related.

30

**Example 21 - Tissue Distribution Of Polypeptide.**

Tissue distribution of mRNA expression of polynucleotides of the present 35 invention is determined using protocols for Northern blot analysis, described by, among others, Sambrook et al. For example, a cDNA probe produced by the method described in Example 20 is labeled with p32 using the rediprime(tm) DNA labeling system (Amersham Life Science), according to manufacturer's instructions. After labeling, the

probe is purified using CHROMA SPIN0-100 column (Clontech Laboratories, Inc.)  
5 according to manufacturer's protocol number PT1200-1. The purified labeled probe is  
then used to examine various tissues for mRNA expression.

10 Tissue Northern blots containing the bound mRNA of various tissues are  
examined with the labeled probe using ExpressHybtm hybridization solution (Clonetech  
according to manufacturers protocol number PT1190-1. Northern blots can be produced  
15 using various protocols well known in the art (e.g., Sambrook et al). Following  
hybridization and washing, the blots are mounted and exposed to film at -70C overnight,  
and the films developed according to standard procedures.

15 **Example 22 - Chromosomal Mapping Of The Polynucleotides.**

An oligonucleotide primer set is designed according to the sequence at the 5' end  
of SEQ ID NO:1 or SEQ ID NO:13 . This primer preferably spans about 100 nucleotides.  
This primer set is then used in a polymerase chain reaction under the following set of  
20 conditions: 30 seconds, 95 degree C; 1 minute, 56 degree C; 1 minute, 70 degree C. This  
cycle is repeated 32 times followed by one 5 minute cycle at 70 degree C. Mammalian  
DNA, preferably human DNA, is used as template in addition to a somatic cell hybrid  
panel containing individual chromosomes or chromosome fragments (Bios, Inc). The  
reactions are analyzed on either 8% polyacrylamide gels or 3.5 % agarose gels.  
25 Chromosome mapping is determined by the presence of an approximately 100 bp PCR  
fragment in the particular somatic cell hybrid.

**Example 23 - Bacterial Expression Of A Polypeptide.**

A polynucleotide encoding a polypeptide of the present invention is amplified  
30 using PCR oligonucleotide primers corresponding to the 5' and 3' ends of the DNA  
sequence, as outlined in Example 20, to synthesize insertion fragments. The primers used  
to amplify the cDNA insert should preferably contain restriction sites, such as BamHI and  
XbaI, at the 5' end of the primers in order to clone the amplified product into the  
35 expression vector. For example, BamHI and XbaI correspond to the restriction enzyme  
sites on the bacterial expression vector pQE-9. (Qiagen, Inc., Chatsworth, CA). This  
plasmid vector encodes antibiotic resistance (Ampr), a bacterial origin of replication (ori),

an IPTG-regulatable promoter/operator (P/O), a ribosome binding site (RBS), a 6-histidine tag (6-His), and restriction enzyme cloning sites.

The pQE-9 vector is digested with BamHI and XbaI and the amplified fragment is ligated into the pQE-9 vector maintaining the reading frame initiated at the bacterial RBS. The ligation mixture is then used to transform the *E. coli* strain M15/rep4 (Qiagen, Inc.) which contains multiple copies of the plasmid pREP4, that expresses the lacI repressor and also confers kanamycin resistance (Kanr). Transformants are identified by their ability to grow on LB plates and ampicillin/kanamycin resistant colonies are selected. Plasmid DNA is isolated and confirmed by restriction analysis.

Clones containing the desired constructs are grown overnight (O/N) in liquid culture in LB media supplemented with both Amp (100 ug/ml) and Kan (25 ug/ml). The O/N culture is used to inoculate a large culture at a ratio of 1:100 to 1:250. The cells are grown to an optical density 600 (O.D.600) of between 0.4 and 0.6. IPTG (Isopropyl-B-D-thiogalacto pyranoside) is then added to a final concentration of 1 mM. IPTG induces by inactivating the lacI repressor, clearing the P/O leading to increased gene expression.

Cells are grown for an extra 3 to 4 hours. Cells are then harvested by centrifugation (20 mins at 6000Xg). The cell pellet is solubilized in the chaotropic agent 6 Molar Guanidine HCl by stirring for 3-4 hours at 4 degree C. The cell debris is removed by centrifugation, and the supernatant containing the polypeptide is loaded onto a nickel-nitrilo-tri-acetic acid ("Ni-NTA") affinity resin column (available from QIAGEN, Inc., supra). Proteins with a 6 x His tag bind to the Ni-NTA resin with high affinity and can be purified in a simple one-step procedure (for details see: The QIAexpressionist (1995) QIAGEN, Inc., supra).

Briefly, the supernatant is loaded onto the column in 6 M guanidine-HCl, pH 8, the column is first washed with 10 volumes of 6 M guanidine-HCl, pH 8, then washed with 10 volumes of 6 M guanidine-HCl pH 6, and finally the polypeptide is eluted with 6 M guanidine-HCl, pH 5.

The purified protein is then renatured by dialyzing it against phosphate-buffered saline (PBS) or 50 mM Na-acetate, pH 6 buffer plus 200 mM NaCl. Alternatively, the protein can be successfully refolded while immobilized on the Ni-NTA column. The recommended conditions are as follows: renature using a linear 6M-1M urea gradient in 500 mM NaCl, 20% glycerol, 20 mM Tris/HCl pH 7.4, containing protease inhibitors.

5 The renaturation should be performed over a period of 1.5 hours or more. After renaturation the proteins are eluted by the addition of 250 mM imidazole. Imidazole is removed by a final dialyzing step against PBS or 50 mM sodium acetate pH 6 buffer plus 200 mM NaCl. The purified protein is stored at 4 degree C or frozen at -80 degree C.

10 **Example 24 - Purification Of A Polypeptide From An Inclusion Body.**

15 The following alternative method can be used to purify a polypeptide expressed in E. coli when it is present in the form of inclusion bodies. Unless otherwise specified, all of the following steps are conducted at 4-10 degree C.

15 Upon completion of the production phase of the E. coli fermentation, the cell culture is cooled to 4-10 degree C and the cells harvested by continuous centrifugation at 15,000 rpm (Heraeus Sepatech). On the basis of the expected yield of protein per unit weight of cell paste and the amount of purified protein required, an appropriate amount of cell paste, by weight, is suspended in a buffer solution containing 100 mM Tris, 50 mM EDTA, pH 7.4. The cells are dispersed to a homogeneous suspension using a high 20 shear mixer.

25 The cells are then lysed by passing the solution through a microfluidizer (Microfluidics, Corp. or APV Gaulin, Inc.) twice at 4000-6000 psi. The homogenate is then mixed with NaCl solution to a final concentration of 0.5 M NaCl, followed by centrifugation at 7000 xg for 15 min. The resultant pellet is washed again using 0.5M NaCl, 100 mM Tris, 50 mM EDTA, pH 7.4.

30 The resulting washed inclusion bodies are solubilized with 1.5 M guanidine hydrochloride (GuHCl) for 2-4 hours. After 7000 xg centrifugation for 15 min., the pellet is discarded and the polypeptide containing supernatant is incubated at 4 degree C overnight to allow further GuHCl extraction.

35 Following high speed centrifugation (30,000 xg) to remove insoluble particles, the GuHCl solubilized protein is refolded by quickly mixing the GuHCl extract with 20 volumes of buffer containing 50 mM sodium, pH 4.5, 150 mM NaCl, 2 mM EDTA by vigorous stirring. The refolded diluted protein solution is kept at 4 degree C without mixing for 12 hours prior to further purification steps.

To clarify the refolded polypeptide solution, a previously prepared tangential filtration unit equipped with 0.16 um membrane filter with appropriate surface area (e.g.,

5 Filtron), equilibrated with 40 mM sodium acetate, pH 6.0 is employed. The filtered sample is loaded onto a cation exchange resin (e.g., Poros HS-50, Perceptive Biosystems). The column is washed with 40 mM sodium acetate, pH 6.0 and eluted with 250 mM, 500 mM, 1000 mM, and 1500 mM NaCl in the same buffer, in a stepwise manner. The absorbance at 280 nm of the effluent is continuously monitored. Fractions are collected and further analyzed by SDS-PAGE.

10 Fractions containing the polypeptide are then pooled and mixed with 4 volumes of water. The diluted sample is then loaded onto a previously prepared set of tandem columns of strong anion (Poros HQ-50, Perceptive Biosystems) and weak anion (Poros CM-20, Perceptive Biosystems) exchange resins. The columns are equilibrated with 40 15 mM sodium acetate, pH 6.0. Both columns are washed with 40 mM sodium acetate, pH 6.0, 200 mM NaCl. The CM-20 column is then eluted using a 10 column volume linear gradient ranging from 0.2 M NaCl, 50 mM sodium acetate, pH 6.0 to 1.0 M NaCl, 50 mM sodium acetate, pH 6.5. Fractions are collected under constant A280 monitoring of the effluent. Fractions containing the polypeptide (determined, for instance, by 16% SDS- 20 PAGE) are then pooled.

25 The resultant polypeptide should exhibit greater than 95% purity after the above refolding and purification steps. No major contaminant bands should be observed from Coomassie blue stained 16% SDS-PAGE gel when 5 ug of purified protein is loaded. The purified protein can also be tested for endotoxin/LPS contamination, and typically the LPS content is less than 0.1 ng/ml according to LAL assays.

**Example 25 - Cloning And Expression Of A Polypeptide In A Baculovirus Expression System.**

30 In this example, the plasmid shuttle vector pAc373 is used to insert a polynucleotide into a baculovirus to express a polypeptide. A typical baculovirus expression vector contains the strong polyhedrin promoter of the *Autographa californica* nuclear polyhedrosis virus (AcMNPV) followed by convenient restriction sites, which may include, for example BamHI, Xba I and Asp718. The polyadenylation site of the 35 simian virus 40 ("SV40") is often used for efficient polyadenylation. For easy selection of recombinant virus, the plasmid contains the beta-galactosidase gene from *E. coli* under control of a weak *Drosophila* promoter in the same orientation, followed by the

polyadenylation signal of the polyhedrin gene. The inserted genes are flanked on both 5 sides by viral sequences for cell-mediated homologous recombination with wild-type viral DNA to generate a viable virus that express the cloned polynucleotide.

Many other baculovirus vectors can be used in place of the vector above, such as 10 pVL941 and pAcIM1, as one skilled in the art would readily appreciate, as long as the construct provides appropriately located signals for transcription, translation, secretion and the like, including a signal peptide and an in-frame AUG as required. Such vectors 15 are described, for instance, in Luckow et al., *Virology* 170:31-39 (1989).

A polynucleotide encoding a polypeptide of the present invention is amplified 20 using PCR oligonucleotide primers corresponding to the 5' and 3' ends of the DNA sequence, as outlined in Example 20, to synthesize insertion fragments. The primers used to amplify the cDNA insert should preferably contain restriction sites at the 5' end of the primers in order to clone the amplified product into the expression vector. Specifically, 25 the cDNA sequence contained in the deposited clone, including the AUG initiation codon and the naturally associated leader sequence identified elsewhere herein (if applicable), is amplified using the PCR protocol described in Example 20. If the naturally occurring signal sequence is used to produce the protein, the vector used does not need a second signal peptide. Alternatively, the vector can be modified to include a baculovirus leader sequence, using the standard methods described in Summers et al., "A Manual of 30 Methods for Baculovirus Vectors and Insect Cell Culture Procedures" Texas Agricultural 35 Experimental Station Bulletin No. 1555 (1987).

The amplified fragment is isolated from a 1% agarose gel using a commercially available kit ("Geneclean" BIO 101 Inc., La Jolla, Ca.). The fragment then is digested with appropriate restriction enzymes and again purified on a 1% agarose gel.

30 The plasmid is digested with the corresponding restriction enzymes and optionally, can be dephosphorylated using calf intestinal phosphatase, using routine procedures known in the art. The DNA is then isolated from a 1% agarose gel using a commercially available kit ("Geneclean" BIO 101 Inc., La Jolla, Ca.).

35 The fragment and the dephosphorylated plasmid are ligated together with T4 DNA ligase. *E. coli* HB101 or other suitable *E. coli* hosts such as XL-1 Blue (Stratagene Cloning Systems, La Jolla, CA) cells are transformed with the ligation mixture and spread on culture plates. Bacteria containing the plasmid are identified by digesting DNA

from individual colonies and analyzing the digestion product by gel electrophoresis. The  
5 sequence of the cloned fragment is confirmed by DNA sequencing.

Five ug of a plasmid containing the polynucleotide is co-transformed with 1.0 ug of a commercially available linearized baculovirus DNA ("BaculoGold™ baculovirus DNA", Pharmingen, San Diego, CA), using the lipofection method described by Felgner et al., Proc. Natl. Acad. Sci. USA 84:7413-7417 (1987). One ug of BaculoGold™ virus  
10 DNA and 5ug of the plasmid are mixed in a sterile well of a microtiter plate containing 50ul of serum-free Grace's medium (Life Technologies Inc., Gaithersburg, MD). Afterwards, 10 ul Lipofectin plus 90 ul Grace's medium are added, mixed and incubated for 15 minutes at room temperature. Then the transfection mixture is added drop-wise to  
15 Sf9 insect cells (ATCC CRL 1711) seeded in a 35 mm tissue culture plate with 1 ml Grace's medium without serum. The plate is then incubated for 5 hours at 27 degrees C. The transfection solution is then removed from the plate and 1 ml of Grace's insect medium supplemented with 10% fetal calf serum is added. Cultivation is then continued at 27 degrees C for four days.

20 After four days the supernatant is collected and a plaque assay is performed, as described by Summers and Smith, *supra*. An agarose gel with "Blue Gal" (Life Technologies Inc., Gaithersburg) is used to allow easy identification and isolation of gal-expressing clones, which produce blue-stained plaques. (A detailed description of a  
25 "plaque assay" of this type can also be found in the user's guide for insect cell culture and baculovirology distributed by Life Technologies Inc., Gaithersburg, page 9-10.) After appropriate incubation, blue stained plaques are picked with the tip of a micropipettor (e.g., Eppendorf). The agar containing the recombinant viruses is then resuspended in a microcentrifuge tube containing 200 ul of Grace's medium and the suspension containing  
30 the recombinant baculovirus is used to infect Sf9 cells seeded in 35 mm dishes. Four days later the supernatants of these culture dishes are harvested and then they are stored at 4 degree C.

35 To verify the expression of the polypeptide, Sf9 cells are grown in Grace's medium supplemented with 10% heat-inactivated FBS. The cells are infected with the recombinant baculovirus containing the polynucleotide at a multiplicity of infection ("MOI") of about 2. If radiolabeled proteins are desired, 6 hours later the medium is removed and is replaced with SF900 II medium minus methionine and cysteine (available

from Life Technologies Inc., Rockville, MD). After 42 hours, 5 uCi of 35S-methionine 5 and 5 uCi 35S-cysteine (available from Amersham) are added. The cells are further incubated for 16 hours and then are harvested by centrifugation. The proteins in the supernatant as well as the intracellular proteins are analyzed by SDS-PAGE followed by autoradiography (if radiolabeled).

10 Microsequencing of the amino acid sequence of the amino terminus of purified protein may be used to determine the amino terminal sequence of the produced protein.

**Example 26 - Expression Of A Polypeptide In Mammalian Cells.**

The polypeptide of the present invention can be expressed in a mammalian cell. 15 A typical mammalian expression vector contains a promoter element, which mediates the initiation of transcription of mRNA, a protein coding sequence, and signals required for the termination of transcription and polyadenylation of the transcript. Additional elements include enhancers, Kozak sequences and intervening sequences flanked by donor and acceptor sites for RNA splicing. Highly efficient transcription is achieved with the early 20 and late promoters from SV40, the long terminal repeats (LTRs) from Retroviruses, e.g., RSV, HTLV, HIVI and the early promoter of the cytomegalovirus (CMV). However, cellular elements can also be used (e.g., the human actin promoter).

Suitable expression vectors for use in practicing the present invention include, for 25 example, vectors such as pSVL and pMSG (Pharmacia, Uppsala, Sweden), pRSVcat (ATCC 37152), pSV2dhfr (ATCC 37146), pBC12MI (ATCC 67109), pCMVSport 2.0, and pCMVSport 3.0. Mammalian host cells that could be used include, human Hela, 293, H9 and Jurkat cells, mouse NIH3T3 and C127 cells, Cos 1, Cos 7 and CV1, quail QC1-3 cells, mouse L cells and Chinese hamster ovary (CHO) cells.

30 Alternatively, the polypeptide can be expressed in stable cell lines containing the polynucleotide integrated into a chromosome. The co-transformation with a selectable marker such as dhfr, gpt, neomycin, hygromycin allows the identification and isolation of the transformed cells.

The transformed gene can also be amplified to express large amounts of the 35 encoded protein. The DHFR (dihydrofolate reductase) marker is useful in developing cell lines that carry several hundred or even several thousand copies of the gene of interest. (See, e.g., Alt, F. W., et al., J. Biol. Chem. 253:1357-1370 (1978); Hamlin, J. L. and Ma,

C., Biochem. et Biophys. Acta, 1097:107-143 (1990); Page, M. J. and Sydenham, M. A.,  
5 Biotechnology 9:64-68 (1991).) Another useful selection marker is the enzyme glutamine  
synthase (GS) (Murphy et al., Biochem J. 227:277-279 (1991); Bebbington et al.,  
Bio/Technology 10:169-175 (1992). Using these markers, the mammalian cells are grown  
in selective medium and the cells with the highest resistance are selected. These cell lines  
contain the amplified gene(s) integrated into a chromosome. Chinese hamster ovary  
10 (CHO) and NSO cells are often used for the production of proteins.

A polynucleotide of the present invention is amplified according to the protocol  
outlined in herein. If the naturally occurring signal sequence is used to produce the  
protein, the vector does not need a second signal peptide. Alternatively, if the naturally  
15 occurring signal sequence is not used, the vector can be modified to include a  
heterologous signal sequence. (See, e.g., WO 96/34891.) The amplified fragment is  
isolated from a 1% agarose gel using a commercially available kit ("Geneclean" BIO 101  
Inc., La Jolla, Ca.). The fragment then is digested with appropriate restriction enzymes  
and again purified on a 1% agarose gel.

20 The amplified fragment is then digested with the same restriction enzyme and  
purified on a 1% agarose gel. The isolated fragment and the dephosphorylated vector are  
then ligated with T4 DNA ligase. E. coli HB101 or XL-1 Blue cells are then transformed  
and bacteria are identified that contain the fragment inserted into plasmid pC6 using, for  
25 instance, restriction enzyme analysis.

Chinese hamster ovary cells lacking an active DHFR gene is used for  
transformation. Five  $\mu$ g of an expression plasmid is cotransformed with 0.5  $\mu$ g of the  
plasmid pSVneo using lipofectin (Felgner et al., supra). The plasmid pSV2-neo contains  
a dominant selectable marker, the neo gene from Tn5 encoding an enzyme that confers  
30 resistance to a group of antibiotics including G418. The cells are seeded in alpha minus  
MEM supplemented with 1 mg/ml G418. After 2 days, the cells are trypsinized and  
seeded in hybridoma cloning plates (Greiner, Germany) in alpha minus MEM  
supplemented with 10, 25, or 50 ng/ml of methotrexate plus 1 mg/ml G418. After about  
35 10-14 days single clones are trypsinized and then seeded in 6-well petri dishes or 10 ml  
flasks using different concentrations of methotrexate (50 nM, 100 nM, 200 nM, 400 nM,  
800 nM). Clones growing at the highest concentrations of methotrexate are then  
transferred to new 6-well plates containing even higher concentrations of methotrexate

(1 uM, 2 uM, 5 uM, 10 mM, 20 mM). The same procedure is repeated until clones are 5 obtained which grow at a concentration of 100 - 200 uM. Expression of the desired gene product is analyzed, for instance, by SDS-PAGE and Western blot or by reversed phase HPLC analysis.

10 **Example 27 – Method of Creating N- and C-terminal Deletion Mutants  
Corresponding to the HGPRBMY1 and HGPRBMY2 Polypeptides of the Present  
Invention.**

As described elsewhere herein, the present invention encompasses the creation of N- and C-terminal deletion mutants, in addition to any combination of N- and C- 15 terminal deletions thereof, corresponding to the HGPRBMY1 or HGPRBMY2 polypeptide of the present invention. A number of methods are available to one skilled in the art for creating such mutants. Such methods may include a combination of PCR amplification and gene cloning methodology. Although one of skill in the art of molecular biology, through the use of the teachings provided or referenced herein, and/or 20 otherwise known in the art as standard methods, could readily create each deletion mutant of the present invention, exemplary methods are described below.

Briefly, using the isolated cDNA clone encoding the full-length HGPRBMY1 or HGPRBMY2 polypeptide sequence (as described herein, for example), appropriate 25 primers of about 15-25 nucleotides derived from the desired 5' and 3' positions of SEQ ID NO:1 or SEQ ID NO:13 may be designed to PCR amplify, and subsequently clone, the intended N- and/or C-terminal deletion mutant. Such primers could comprise, for example, an initiation and stop codon for the 5' and 3' primer, respectively. Such primers may also comprise restriction sites to facilitate cloning of the deletion mutant 30 post amplification. Moreover, the primers may comprise additional sequences, such as, for example, flag-tag sequences, kozac sequences, or other sequences discussed and/or referenced herein.

For example, in the case of the M76 to H431 HGPRBMY2 N-terminal deletion 35 mutant, the following primers could be used to amplify a cDNA fragment corresponding to this deletion mutant:

5	5' Primer	5'-GCAGCA <u>GC</u> GGCCGC ATGCGCACCGTCACCAACATC -3' (SEQ ID NO:20) <i>NotI</i>
10	3' Primer	5'- GCAGCA <u>GTC</u> GAC ATGCCCACTGTCTAAAGGAGAATT C -3' (SEQ ID NO:21) <i>Sall</i>

For example, in the case of the M1 to Y305 HGPRBMY2 C-terminal deletion mutant, the following primers could be used to amplify a cDNA fragment corresponding to this deletion mutant:

15	5' Primer	5'- GCAGCA <u>GC</u> GGCCGC CGGCGCATGGGGCCCAGATCCCCG -3' (SEQ ID NO:22) <i>NotI</i>
20	3' Primer	5'- GCAGCA <u>GTC</u> GAC GAACACACTCTCCTGCCTCTGGAGG -3' (SEQ ID NO:23) <i>Sall</i>

For example, in the case of the R50 to F359 HGPRBMY1 N-terminal deletion mutant, the following primers could be used to amplify a cDNA fragment corresponding to this deletion mutant:

30	5' Primer	5'-GCAGCA <u>GC</u> GGCCGC ATGCAGGTCCC AACAGCACCGGCC -3' (SEQ ID NO:52) <i>NotI</i>
	3' Primer	5'- GCAGCA <u>GTC</u> GAC CTTGTACACGTGGTAGTAGCTTTG -3' (SEQ ID NO:53) <i>Sall</i>

35 For example, in the case of the M1 to K276 HGPRBMY1 C-terminal deletion mutant, the following primers could be used to amplify a cDNA fragment corresponding to this deletion mutant:

5	5' Primer	5' - GCAGCA <u>GC</u> GGCCGC ATGCAGGCGCTAACATTACCCGG -3' (SEQ ID NO:54) <i>NotI</i>
10	3' Primer	5' - GCAGCA <u>GT</u> CGAC ATATTCCCTTCAAAATTACTG -3' (SEQ ID NO:55) <i>Sall</i>

Representative PCR amplification conditions are provided below, although the skilled artisan would appreciate that other conditions may be required for efficient amplification. A 100 ul PCR reaction mixture may be prepared using 10ng of the template DNA (cDNA clone of HGPRBMY2), 200 uM 4dNTPs, 1uM primers, 0.25U Taq DNA polymerase (PE), and standard Taq DNA polymerase buffer. Typical PCR cycling condition are as follows:

20

20-25 cycles: 45 sec, 93 degrees

2 min, 50 degrees

2 min, 72 degrees

25 1 cycle: 10 min, 72 degrees

After the final extension step of PCR, 5U Klenow Fragment may be added and incubated for 15 min at 30 degrees.

Upon digestion of the fragment with the *NotI* and *Sall* restriction enzymes, the fragment could be cloned into an appropriate expression and/or cloning vector which has been similarly digested (e.g., pSport1, among others). The skilled artisan would appreciate that other plasmids could be equally substituted, and may be desirable in certain circumstances. The digested fragment and vector are then ligated using a DNA ligase, and then used to transform competent *E.coli* cells using methods provided herein and/or otherwise known in the art.

The 5' primer sequence for amplifying any additional N-terminal deletion mutants may be determined by reference to the following formula:

(S+(X \* 3)) to ((S+(X \* 3))+25), wherein 'S' is equal to the nucleotide position 5 of the initiating start codon of the HGPRBMY1 or HGPRBMY2 gene (SEQ ID NO:1 or SEQ ID NO:13 ), and 'X' is equal to the most N-terminal amino acid of the intended N-terminal deletion mutant. The first term will provide the start 5' nucleotide position of the 5' primer, while the second term will provide the end 3' nucleotide position of the 5' primer corresponding to sense strand of SEQ ID NO:1 or SEQ ID NO:13 . Once the 10 corresponding nucleotide positions of the primer are determined, the final nucleotide sequence may be created by the addition of applicable restriction site sequences to the 5' end of the sequence, for example. As referenced herein, the addition of other sequences to the 5' primer may be desired in certain circumstances (e.g., kozac sequences, etc.).

15 The 3' primer sequence for amplifying any additional N-terminal deletion mutants may be determined by reference to the following formula:

(S+(X \* 3)) to ((S+(X \* 3))-25), wherein 'S' is equal to the nucleotide position of the initiating start codon of the HGPRBMY1 or HGPRBMY2 gene (SEQ ID NO:1 or SEQ ID NO:13 ), and 'X' is equal to the most C-terminal amino acid of the intended N-terminal deletion mutant. The first term will provide the start 5' nucleotide position of the 3' primer, while the second term will provide the end 3' nucleotide position of the 3' primer corresponding to the anti-sense strand of SEQ ID NO:1 or SEQ ID NO:13 . Once the 20 corresponding nucleotide positions of the primer are determined, the final nucleotide sequence may be created by the addition of applicable restriction site sequences to the 5' end of the sequence, for example. As referenced herein, the addition of other sequences to the 3' primer may be desired in certain circumstances (e.g., stop codon sequences, etc.). The skilled artisan would appreciate that modifications of the above nucleotide 25 positions may be necessary for optimizing PCR amplification.

30 The same general formulas provided above may be used in identifying the 5' and 3' primer sequences for amplifying any C-terminal deletion mutant of the present invention. Moreover, the same general formulas provided above may be used in identifying the 5' and 3' primer sequences for amplifying any combination of N-terminal and C-terminal deletion mutant of the present invention. The skilled artisan would 35 appreciate that modifications of the above nucleotide positions may be necessary for optimizing PCR amplification.

5   **Example 28 - Protein Fusions.**

The polypeptides of the present invention are preferably fused to other proteins. These fusion proteins can be used for a variety of applications. For example, fusion of the present polypeptides to His-tag, HA-tag, protein A, IgG domains, and maltose binding protein facilitates purification. (See Example described herein; see also EP A 394,827; 10 Traunecker, et al., *Nature* 331:84-86 (1988).) Similarly, fusion to IgG-1, IgG-3, and albumin increases the half-life time in vivo. Nuclear localization signals fused to the polypeptides of the present invention can target the protein to a specific subcellular localization, while covalent heterodimer or homodimers can increase or decrease the 15 activity of a fusion protein. Fusion proteins can also create chimeric molecules having more than one function. Finally, fusion proteins can increase solubility and/or stability of the fused protein compared to the non-fused protein. All of the types of fusion proteins described above can be made by modifying the following protocol, which outlines the fusion of a polypeptide to an IgG molecule.

20   Briefly, the human Fc portion of the IgG molecule can be PCR amplified, using primers that span the 5' and 3' ends of the sequence described below. These primers also should have convenient restriction enzyme sites that will facilitate cloning into an expression vector, preferably a mammalian expression vector. Note that the 25 polynucleotide is cloned without a stop codon, otherwise a fusion protein will not be produced.

The naturally occurring signal sequence may be used to produce the protein (if applicable). Alternatively, if the naturally occurring signal sequence is not used, the vector can be modified to include a heterologous signal sequence. (See, e.g., WO 30 96/34891 and/or US Patent No. 6,066,781, *supra*.)

Human IgG Fc region:

35   GGGATCCGGAGCCAAATCTTCTGACAAAACACACATGCCACCGT  
GCCAGCACCTGAATTCGAGGGTGACCGTCAGTCTTCCCTCTCCCCCAAAA  
CCCCAGGACACCCCTCATGATCTCCGGACTCCTGAGGTACATGCGTGGTGG  
TGGACGTAAGCCACGAAGACCCCTGAGGTCAAGTTCAACTGGTACGTGGACGG  
CGTGGAGGTGCATAATGCCAAGACAAAGCCGCGGGAGGAGCAGTACAACAG

CACGTACCGTGTGGTCAGCGCCTCACCGCCTGCACCAGGACTGGCTGAAT  
5 GGCAAGGAGTACAAGTGCAAGGTCTCCAACAAAGCCCTCCCAACCCCCATCG  
AGAAAACCATCTCCAAAGCCAAGGGCAGCCCCGAGAACCAAGGACAGGTGTACA  
CCCTGCCCTCATCCGGATGAGCTGACCAAGAACCAAGGTCAGCCTGACCTG  
CCTGGTCAAAGGCTTCTATCCAAGCGACATCGCCGTGGAGTGGAGAGCAAT  
10 GGGCAGCCGGAGAACAACTACAAGACCACGCCCTCCCGTGTGGACTCCGACG  
GCTCCITCTCCTCTACAGCAAGCTACCGTGGACAAGAGCAGGTGGCAGCA  
GGGAAACGTCTTCTCATGCTCCGTATGCATGAGGCTCTGCACAACCACACTAC  
ACCGAGAACAGAGCCTCTCCCTGTCTCCGGTAAATGAGTGCACGGCCGCGAC  
TCTAGAGGAT (SEQ ID NO:24)

15

**Example 29 - Production Of An Antibody From A Polypeptide.**

The antibodies of the present invention can be prepared by a variety of methods. (See, Current Protocols, Chapter 2.) As one example of such methods, cells expressing a polypeptide of the present invention are administered to an animal to induce the 20 production of sera containing polyclonal antibodies. In a preferred method, a preparation of the protein is prepared and purified to render it substantially free of natural contaminants. Such a preparation is then introduced into an animal in order to produce polyclonal antisera of greater specific activity.

25 In the most preferred method, the antibodies of the present invention are monoclonal antibodies (or protein binding fragments thereof). Such monoclonal antibodies can be prepared using hybridoma technology. (Köhler et al., Nature 256:495 (1975); Köhler et al., Eur. J. Immunol. 6:511 (1976); Köhler et al., Eur. J. Immunol. 6:292 (1976); Hammerling et al., in: Monoclonal Antibodies and T-Cell Hybridomas, 30 Elsevier, N.Y., pp. 563-681 (1981).) In general, such procedures involve immunizing an animal (preferably a mouse) with polypeptide or, more preferably, with a polypeptide-expressing cell. Such cells may be cultured in any suitable tissue culture medium; however, it is preferable to culture cells in Earle's modified Eagle's medium 35 supplemented with 10% fetal bovine serum (inactivated at about 56 degrees C), and supplemented with about 10 g/l of nonessential amino acids, about 1,000 U/ml of penicillin, and about 100 ug/ml of streptomycin.

The splenocytes of such mice are extracted and fused with a suitable myeloma 5 cell line. Any suitable myeloma cell line may be employed in accordance with the present invention; however, it is preferable to employ the parent myeloma cell line (SP2O), available from the ATCC. After fusion, the resulting hybridoma cells are selectively maintained in HAT medium, and then cloned by limiting dilution as described by Wands et al. (Gastroenterology 80:225-232 (1981).) The hybridoma cells obtained through such 10 a selection are then assayed to identify clones which secrete antibodies capable of binding the polypeptide.

Alternatively, additional antibodies capable of binding to the polypeptide can be produced in a two-step procedure using anti-idiotypic antibodies. Such a method makes 15 use of the fact that antibodies are themselves antigens, and therefore, it is possible to obtain an antibody that binds to a second antibody. In accordance with this method, protein specific antibodies are used to immunize an animal, preferably a mouse. The splenocytes of such an animal are then used to produce hybridoma cells, and the hybridoma cells are screened to identify clones that produce an antibody whose ability 20 to bind to the protein-specific antibody can be blocked by the polypeptide. Such antibodies comprise anti-idiotypic antibodies to the protein-specific antibody and can be used to immunize an animal to induce formation of further protein-specific antibodies.

It will be appreciated that Fab and F(ab')2 and other fragments of the antibodies 25 of the present invention may be used according to the methods disclosed herein. Such fragments are typically produced by proteolytic cleavage, using enzymes such as papain (to produce Fab fragments) or pepsin (to produce F(ab')2 fragments). Alternatively, protein-binding fragments can be produced through the application of recombinant DNA technology or through synthetic chemistry.

30 For in vivo use of antibodies in humans, it may be preferable to use "humanized" chimeric monoclonal antibodies. Such antibodies can be produced using genetic constructs derived from hybridoma cells producing the monoclonal antibodies described above. Methods for producing chimeric antibodies are known in the art. (See, for review, Morrison, Science 229:1202 (1985); Oi et al., BioTechniques 4:214 (1986); Cabilly et 35 al., U.S. Patent No. 4,816,567; Taniguchi et al., EP 171496; Morrison et al., EP 173494; Neuberger et al., WO 8601533; Robinson et al., WO 8702671; Boulian et al., Nature 312:643 (1984); Neuberger et al., Nature 314:268 (1985).)

Moreover, in another preferred method, the antibodies directed against the 5 polypeptides of the present invention may be produced in plants. Specific methods are disclosed in US Patent Nos. 5,959,177, and 6,080,560, which are hereby incorporated in their entirety herein. The methods not only describe methods of expressing antibodies, but also the means of assembling foreign multimeric proteins in plants (i.e., antibodies, etc.), and the subsequent secretion of such antibodies from the plant.

10

**Example 26 - Method Of Enhancing The Biological Activity/Functional Characteristics Of Invention Through Molecular Evolution.**

Although many of the most biologically active proteins known are highly 15 effective for their specified function in an organism, they often possess characteristics that make them undesirable for transgenic, therapeutic, and/or industrial applications. Among these traits, a short physiological half-life is the most prominent problem, and is present either at the level of the protein, or the level of the proteins mRNA. The ability to extend the half-life, for example, would be particularly important for a proteins use in 20 gene therapy, transgenic animal production, the bioprocess production and purification of the protein, and use of the protein as a chemical modulator among others. Therefore, there is a need to identify novel variants of isolated proteins possessing characteristics which enhance their application as a therapeutic for treating diseases of animal origin, in 25 addition to the proteins applicability to common industrial and pharmaceutical applications.

Thus, one aspect of the present invention relates to the ability to enhance specific characteristics of invention through directed molecular evolution. Such an enhancement may, in a non-limiting example, benefit the inventions utility as an essential component 30 in a kit, the inventions physical attributes such as its solubility, structure, or codon optimization, the inventions specific biological activity, including any associated enzymatic activity, the proteins enzyme kinetics, the proteins Ki, Kcat, Km, Vmax, Kd, protein-protein activity, protein-DNA binding activity, antagonist/inhibitory activity 35 (including direct or indirect interaction), agonist activity (including direct or indirect interaction), the proteins antigenicity (e.g., where it would be desirable to either increase or decrease the antigenic potential of the protein), the immunogenicity of the protein, the ability of the protein to form dimers, trimers, or multimers with either itself or other

proteins, the antigenic efficacy of the invention, including its subsequent use a  
5 preventative treatment for disease or disease states, or as an effector for targeting diseased  
genes. Moreover, the ability to enhance specific characteristics of a protein may also be  
applicable to changing the characterized activity of an enzyme to an activity completely  
unrelated to its initially characterized activity. Other desirable enhancements of the  
10 invention would be specific to each individual protein, and would thus be well known in  
the art and contemplated by the present invention.

For example, an engineered G-protein coupled receptor may be constitutively active upon binding of its cognate ligand. Alternatively, an engineered G-protein coupled receptor may be constitutively active in the absence of ligand binding. In yet another  
15 example, an engineered GPCR may be capable of being activated with less than all of the regulatory factors and/or conditions typically required for GPCR activation (e.g., ligand binding, phosphorylation, conformational changes, etc.). Such GPCRs would be useful in screens to identify GPCR modulators, among other uses described herein.

Directed evolution is comprised of several steps. The first step is to establish a  
20 library of variants for the gene or protein of interest. The most important step is to then select for those variants that entail the activity you wish to identify. The design of the screen is essential since your screen should be selective enough to eliminate non-useful variants, but not so stringent as to eliminate all variants. The last step is then to repeat the  
25 above steps using the best variant from the previous screen. Each successive cycle, can then be tailored as necessary, such as increasing the stringency of the screen, for example.

Over the years, there have been a number of methods developed to introduce mutations into macromolecules. Some of these methods include, random mutagenesis, “error-prone” PCR, chemical mutagenesis, site-directed mutagenesis, and other methods  
30 well known in the art (for a comprehensive listing of current mutagenesis methods, see Maniatis, Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Press, Cold Spring, NY (1982)). Typically, such methods have been used, for example, as tools for identifying the core functional region(s) of a protein or the function of specific domains  
35 of a protein (if a multi-domain protein). However, such methods have more recently been applied to the identification of macromolecule variants with specific or enhanced characteristics.

Random mutagenesis has been the most widely recognized method to date.

Typically, this has been carried out either through the use of "error-prone" PCR (as 5 described in Moore, J., et al, *Nature Biotechnology* 14:458, (1996), or through the application of randomized synthetic oligonucleotides corresponding to specific regions of interest (as described by Derbyshire, K.M. et al, *Gene*, 46:145-152, (1986), and Hill, 10 DE, et al, *Methods Enzymol.*, 55:559-568, (1987). Both approaches have limits to the level of mutagenesis that can be obtained. However, either approach enables the 15 investigator to effectively control the rate of mutagenesis. This is particularly important considering the fact that mutations beneficial to the activity of the enzyme are fairly rare. In fact, using too high a level of mutagenesis may counter or inhibit the desired benefit of a useful mutation.

15 While both of the aforementioned methods are effective for creating randomized pools of macromolecule variants, a third method, termed "DNA Shuffling", or "sexual PCR" (WPC, Stemmer, *PNAS*, 91:10747, (1994)) has recently been elucidated. DNA shuffling has also been referred to as "directed molecular evolution", "exon-shuffling", "directed enzyme evolution", "in vitro evolution", and "artificial evolution". Such 20 reference terms are known in the art and are encompassed by the invention. This new, preferred, method apparently overcomes the limitations of the previous methods in that it not only propagates positive traits, but simultaneously eliminates negative traits in the resulting progeny.

25 DNA shuffling accomplishes this task by combining the principal of in vitro recombination, along with the method of "error-prone" PCR. In effect, you begin with a randomly digested pool of small fragments of your gene, created by Dnase I digestion, and then introduce said random fragments into an "error-prone" PCR assembly reaction. During the PCR reaction, the randomly sized DNA fragments not only hybridize to their 30 cognate strand, but also may hybridize to other DNA fragments corresponding to different regions of the polynucleotide of interest – regions not typically accessible via hybridization of the entire polynucleotide. Moreover, since the PCR assembly reaction utilizes "error-prone" PCR reaction conditions, random mutations are introduced during the DNA synthesis step of the PCR reaction for all of the fragments -further diversifying 35 the potential hybridization sites during the annealing step of the reaction.

A variety of reaction conditions could be utilized to carry-out the DNA shuffling reaction. However, specific reaction conditions for DNA shuffling are provided, for

example, in PNAS, 91:10747, (1994). Briefly:

5        Prepare the DNA substrate to be subjected to the DNA shuffling reaction. Preparation may be in the form of simply purifying the DNA from contaminating cellular material, chemicals, buffers, oligonucleotide primers, deoxynucleotides, RNAs, etc., and may entail the use of DNA purification kits as those provided by Qiagen, Inc., or by the Promega, Corp., for example.

10      Once the DNA substrate has been purified, it would be subjected to Dnase I digestion. About 2-4ug of the DNA substrate(s) would be digested with .0015 units of Dnase I (Sigma) per ul in 100ul of 50mM Tris-HCL, pH 7.4/1mM MgCl<sub>2</sub> for 10-20 min. at room temperature. The resulting fragments of 10-50bp could then be purified by

15      running them through a 2% low-melting point agarose gel by electrophoresis onto DE81 ion-exchange paper (Whatmann) or could be purified using Microcon concentrators (Amicon) of the appropriate molecular weight cutoff, or could use oligonucleotide purification columns (Qiagen), in addition to other methods known in the art. If using DE81 ion-exchange paper, the 10-50bp fragments could be eluted from said paper using

20      1M NaCl, followed by ethanol precipitation.

The resulting purified fragments would then be subjected to a PCR assembly reaction by re-suspension in a PCR mixture containing: 2mM of each dNTP, 2.2mM MgCl<sub>2</sub>, 50 mM KCl, 10mM Tris•HCL, pH 9.0, and 0.1% Triton X-100, at a final 25 fragment concentration of 10-30ng/ul. No primers are added at this point. *Taq* DNA polymerase (Promega) would be used at 2.5 units per 100ul of reaction mixture. A PCR program of 94 C for 60s; 94 C for 30s, 50-55 C for 30s, and 72 C for 30s using 30-45 cycles, followed by 72 C for 5min using an MJ Research (Cambridge, MA) PTC-150 thermocycler. After the assembly reaction is completed, a 1:40 dilution of the resulting 30 primerless product would then be introduced into a PCR mixture (using the same buffer mixture used for the assembly reaction) containing 0.8um of each primer and subjecting this mixture to 15 cycles of PCR (using 94 C for 30s, 50 C for 30s, and 72 C for 30s). The referred primers would be primers corresponding to the nucleic acid sequences of the 35 polynucleotide(s) utilized in the shuffling reaction. Said primers could consist of modified nucleic acid base pairs using methods known in the art and referred to elsewhere herein, or could contain additional sequences (i.e., for adding restriction sites, mutating specific base-pairs, etc.).

The resulting shuffled, assembled, and amplified product can be purified using 5 methods well known in the art (e.g., Qiagen PCR purification kits) and then subsequently cloned using appropriate restriction enzymes.

Although a number of variations of DNA shuffling have been published to date, such variations would be obvious to the skilled artisan and are encompassed by the 10 invention. The DNA shuffling method can also be tailored to the desired level of mutagenesis using the methods described by Zhao, et al. (Nucl Acid Res., 25(6):1307-1308, (1997).

As described above, once the randomized pool has been created, it can then be subjected to a specific screen to identify the variant possessing the desired 15 characteristic(s). Once the variant has been identified, DNA corresponding to the variant could then be used as the DNA substrate for initiating another round of DNA shuffling. This cycle of shuffling, selecting the optimized variant of interest, and then re-shuffling, can be repeated until the ultimate variant is obtained. Examples of model screens applied to identify variants created using DNA shuffling technology may be found in the 20 following publications: J. C., Moore, et al., J. Mol. Biol., 272:336-347, (1997), F.R., Cross, et al., Mol. Cell. Biol., 18:2923-2931, (1998), and A. Cramer., et al., Nat. Biotech., 15:436-438, (1997).

DNA shuffling has several advantages. First, it makes use of beneficial mutations. 25 When combined with screening, DNA shuffling allows the discovery of the best mutational combinations and does not assume that the best combination contains all the mutations in a population. Secondly, recombination occurs simultaneously with point mutagenesis. An effect of forcing DNA polymerase to synthesize full-length genes from the small fragment DNA pool is a background mutagenesis rate. In combination with a 30 stringent selection method, enzymatic activity has been evolved up to 16000 fold increase over the wild-type form of the enzyme. In essence, the background mutagenesis yielded the genetic variability on which recombination acted to enhance the activity.

A third feature of recombination is that it can be used to remove deleterious 35 mutations. As discussed above, during the process of the randomization, for every one beneficial mutation, there may be at least one or more neutral or inhibitory mutations. Such mutations can be removed by including in the assembly reaction an excess of the wild-type random-size fragments, in addition to the random-size fragments of the

selected mutant from the previous selection. During the next selection, some of the most 5 active variants of the polynucleotide/polypeptide/enzyme, should have lost the inhibitory mutations.

Finally, recombination enables parallel processing. This represents a significant advantage since there are likely multiple characteristics that would make a protein more desirable (e.g. solubility, activity, etc.). Since it is increasingly difficult to screen for more 10 than one desirable trait at a time, other methods of molecular evolution tend to be inhibitory. However, using recombination, it would be possible to combine the randomized fragments of the best representative variants for the various traits, and then select for multiple properties at once.

15 DNA shuffling can also be applied to the polynucleotides and polypeptides of the present invention to decrease their immunogenicity in a specified host. For example, a particular variant of the present invention may be created and isolated using DNA shuffling technology. Such a variant may have all of the desired characteristics, though may be highly immunogenic in a host due to its novel intrinsic structure. Specifically, the 20 desired characteristic may cause the polypeptide to have a non-native structure which could no longer be recognized as a "self" molecule, but rather as a "foreign", and thus activate a host immune response directed against the novel variant. Such a limitation can be overcome, for example, by including a copy of the gene sequence for a xenobiotic 25 ortholog of the native protein in with the gene sequence of the novel variant gene in one or more cycles of DNA shuffling. The molar ratio of the ortholog and novel variant DNAs could be varied accordingly. Ideally, the resulting hybrid variant identified would contain at least some of the coding sequence which enabled the xenobiotic protein to evade the host immune system, and additionally, the coding sequence of the original 30 novel variant that provided the desired characteristics.

Likewise, the invention encompasses the application of DNA shuffling technology to the evolution of polynucleotides and polypeptides of the invention, wherein one or more cycles of DNA shuffling include, in addition to the gene template DNA, 35 oligonucleotides coding for known allelic sequences, optimized codon sequences, known variant sequences, known polynucleotide polymorphism sequences, known ortholog sequences, known homologue sequences, additional homologous sequences, additional non-homologous sequences, sequences from another species, and any number and

combination of the above.

5 In addition to the described methods above, there are a number of related methods that may also be applicable, or desirable in certain cases. Representative among these are the methods discussed in PCT applications WO 98/31700, and WO 98/32845, which are hereby incorporated by reference. Furthermore, related methods can also be applied to the 10 polynucleotide sequences of the present invention in order to evolve invention for creating ideal variants for use in gene therapy, protein engineering, evolution of whole cells containing the variant, or in the evolution of entire enzyme pathways containing polynucleotides of the invention as described in PCT applications WO 98/13485, WO 98/13487, WO 98/27230, WO 98/31837, and Crameri, A., et al., *Nat. Biotech.*, 15:436-15 438, (1997), respectively.

Additional methods of applying "DNA Shuffling" technology to the polynucleotides and polypeptides of the present invention, including their proposed applications, may be found in US Patent No. 5,605,793; PCT Application No. WO 95/22625; PCT Application No. WO 97/20078; PCT Application No. WO 97/35966; and 20 PCT Application No. WO 98/42832; PCT Application No. WO 00/09727 specifically provides methods for applying DNA shuffling to the identification of herbicide selective crops which could be applied to the polynucleotides and polypeptides of the present invention; additionally, PCT Application No. WO 00/12680 provides methods and 25 compositions for generating, modifying, adapting, and optimizing polynucleotide sequences that confer detectable phenotypic properties on plant species; each of the above are hereby incorporated in their entirety herein for all purposes.

**Example 30 - Method Of Determining Alterations In A Gene Corresponding To A 30 Polynucleotide.**

RNA isolated from entire families or individual patients presenting with a phenotype of interest (such as a disease) is be isolated. cDNA is then generated from these RNA samples using protocols known in the art. (See, Sambrook.) The cDNA is then used as a template for PCR, employing primers surrounding regions of interest in 35 SEQ ID NO:1. Suggested PCR conditions consist of 35 cycles at 95 degrees C for 30 seconds; 60-120 seconds at 52-58 degrees C; and 60-120 seconds at 70 degrees C, using buffer solutions described in Sidransky et al., *Science* 252:706 (1991).

PCR products are then sequenced using primers labeled at their 5' end with T4 5 polynucleotide kinase, employing SequiTherm Polymerase. (Epicentre Technologies). The intron-exon borders of selected exons is also determined and genomic PCR products analyzed to confirm the results. PCR products harboring suspected mutations is then cloned and sequenced to validate the results of the direct sequencing.

10 PCR products is cloned into T-tailed vectors as described in Holton et al., Nucleic Acids Research, 19:1156 (1991) and sequenced with T7 polymerase (United States Biochemical). Affected individuals are identified by mutations not present in unaffected individuals.

15 Genomic rearrangements are also observed as a method of determining alterations in a gene corresponding to a polynucleotide. Genomic clones isolated according to Example 2 are nick-translated with digoxigenin-deoxy-uridine 5'-triphosphate (Boehringer Manheim), and FISH performed as described in Johnson et al., Methods Cell Biol. 35:73-99 (1991). Hybridization with the labeled probe is carried out using a vast excess of human cot-1 DNA for specific hybridization to the corresponding genomic locus.

20 Chromosomes are counterstained with 4,6-diamino-2-phenylidole and propidium iodide, producing a combination of C- and R-bands. Aligned images for precise mapping are obtained using a triple-band filter set (Chroma Technology, Brattleboro, VT) in combination with a cooled charge-coupled device camera (Photometrics, Tucson, AZ) 25 and variable excitation wavelength filters. (Johnson et al., Genet. Anal. Tech. Appl., 8:75 (1991).) Image collection, analysis and chromosomal fractional length measurements are performed using the ISee Graphical Program System. (Inovision Corporation, Durham, NC.) Chromosome alterations of the genomic region hybridized by the probe are identified as insertions, deletions, and translocations. These alterations are used as a 30 diagnostic marker for an associated disease.

**Example 31 - Method Of Detecting Abnormal Levels Of A Polypeptide In A Biological Sample.**

35 A polypeptide of the present invention can be detected in a biological sample, and if an increased or decreased level of the polypeptide is detected, this polypeptide is a marker for a particular phenotype. Methods of detection are numerous, and thus, it is understood that one skilled in the art can modify the following assay to fit their particular

needs.

5 For example, antibody-sandwich ELISAs are used to detect polypeptides in a sample, preferably a biological sample. Wells of a microtiter plate are coated with specific antibodies, at a final concentration of 0.2 to 10  $\mu$ g/ml. The antibodies are either monoclonal or polyclonal and are produced by the method described elsewhere herein. The wells are blocked so that non-specific binding of the polypeptide to the well is  
10 reduced.

The coated wells are then incubated for > 2 hours at RT with a sample containing the polypeptide. Preferably, serial dilutions of the sample should be used to validate results. The plates are then washed three times with deionized or distilled water to  
15 remove unbounded polypeptide.

Next, 50  $\mu$ l of specific antibody-alkaline phosphatase conjugate, at a concentration of 25-400 ng, is added and incubated for 2 hours at room temperature. The plates are again washed three times with deionized or distilled water to remove unbounded conjugate.

20 Add 75  $\mu$ l of 4-methylumbelliferyl phosphate (MUP) or p-nitrophenyl phosphate (NPP) substrate solution to each well and incubate 1 hour at room temperature. Measure the reaction by a microtiter plate reader. Prepare a standard curve, using serial dilutions of a control sample, and plot polypeptide concentration on the X-axis (log scale) and fluorescence or absorbance of the Y-axis (linear scale). Interpolate the concentration of  
25 the polypeptide in the sample using the standard curve.

#### **Example 32 – Formulation.**

The invention also provides methods of treatment and/or prevention diseases, disorders, and/or conditions (such as, for example, any one or more of the diseases or disorders disclosed herein) by administration to a subject of an effective amount of a Therapeutic. By therapeutic is meant a polynucleotides or polypeptides of the invention (including fragments and variants), agonists or antagonists thereof, and/or antibodies thereto, in combination with a pharmaceutically acceptable carrier type (e.g., a sterile  
35 carrier).

The Therapeutic will be formulated and dosed in a fashion consistent with good medical practice, taking into account the clinical condition of the individual patient

(especially the side effects of treatment with the Therapeutic alone), the site of delivery, 5 the method of administration, the scheduling of administration, and other factors known to practitioners. The "effective amount" for purposes herein is thus determined by such considerations.

As a general proposition, the total pharmaceutically effective amount of the Therapeutic administered parenterally per dose will be in the range of about 1ug/kg/day 10 to 10 mg/kg/day of patient body weight, although, as noted above, this will be subject to therapeutic discretion. More preferably, this dose is at least 0.01 mg/kg/day, and most preferably for humans between about 0.01 and 1 mg/kg/day for the hormone. If given continuously, the Therapeutic is typically administered at a dose rate of about 1 15 ug/kg/hour to about 50 ug/kg/hour, either by 1-4 injections per day or by continuous subcutaneous infusions, for example, using a mini-pump. An intravenous bag solution may also be employed. The length of treatment needed to observe changes and the interval following treatment for responses to occur appears to vary depending on the desired effect.

20 Therapeutics can be administered orally, rectally, parenterally, intracisternally, intravaginally, intraperitoneally, topically (as by powders, ointments, gels, drops or transdermal patch), buccally, or as an oral or nasal spray. "Pharmaceutically acceptable carrier" refers to a non-toxic solid, semisolid or liquid filler, diluent, encapsulating 25 material or formulation auxiliary of any. The term "parenteral" as used herein refers to modes of administration which include intravenous, intramuscular, intraperitoneal, intrasternal, subcutaneous and intraarticular injection and infusion.

Therapeutics of the invention are also suitably administered by sustained-release 30 systems. Suitable examples of sustained-release Therapeutics are administered orally, rectally, parenterally, intracisternally, intravaginally, intraperitoneally, topically (as by powders, ointments, gels, drops or transdermal patch), buccally, or as an oral or nasal spray. "Pharmaceutically acceptable carrier" refers to a non-toxic solid, semisolid or liquid filler, diluent, encapsulating 35 material or formulation auxiliary of any type. The term "parenteral" as used herein refers to modes of administration which include intravenous, intramuscular, intraperitoneal, intrasternal, subcutaneous and intraarticular injection and infusion.

Therapeutics of the invention may also be suitably administered by sustained-

5 release systems. Suitable examples of sustained-release Therapeutics include suitable polymeric materials (such as, for example, semi-permeable polymer matrices in the form of shaped articles, e.g., films, or microcapsules), suitable hydrophobic materials (for example as an emulsion in an acceptable oil) or ion exchange resins, and sparingly soluble derivatives (such as, for example, a sparingly soluble salt).

10 Sustained-release matrices include polylactides (U.S. Pat. No. 3,773,919, EP 58,481), copolymers of L-glutamic acid and gamma-ethyl-L-glutamate (Sidman et al., Biopolymers 22:547-556 (1983)), poly (2- hydroxyethyl methacrylate) (Langer et al., J. Biomed. Mater. Res. 15:167-277 (1981), and Langer, Chem. Tech. 12:98-105 (1982)), ethylene vinyl acetate (Langer et al., Id.) or poly-D- (-)-3-hydroxybutyric acid (EP 15 133,988).

15 Sustained-release Therapeutics also include liposomally entrapped Therapeutics of the invention (see, generally, Langer, Science 249:1527-1533 (1990); Treat et al., in Liposomes in the Therapy of Infectious Disease and Cancer, Lopez-Berestein and Fidler (eds.), Liss, New York, pp. 317 -327 and 353-365 (1989)). Liposomes containing the 20 Therapeutic are prepared by methods known per se: DE 3,218,121; Epstein et al., Proc. Natl. Acad. Sci. (USA) 82:3688-3692 (1985); Hwang et al., Proc. Natl. Acad. Sci.(USA) 77:4030-4034 (1980); EP 52,322; EP 36,676; EP 88,046; EP 143,949; EP 142,641; Japanese Pat. Appl. 83-118008; U.S. Pat. Nos. 4,485,045 and 4,544,545; and EP 102,324. 25 Ordinarily, the liposomes are of the small (about 200-800 Angstroms) unilamellar type in which the lipid content is greater than about 30 mol. percent cholesterol, the selected proportion being adjusted for the optimal Therapeutic.

30 In yet an additional embodiment, the Therapeutics of the invention are delivered by way of a pump (see Langer, *supra*; Sefton, CRC Crit. Ref. Biomed. Eng. 14:201 (1987); Buchwald et al., Surgery 88:507 (1980); Saudek et al., N. Engl. J. Med. 321:574 (1989)).

35 Other controlled release systems are discussed in the review by Langer (Science 249:1527-1533 (1990)).

For parenteral administration, in one embodiment, the Therapeutic is formulated generally by mixing it at the desired degree of purity, in a unit dosage injectable form (solution, suspension, or emulsion), with a pharmaceutically acceptable carrier, i.e., one that is non-toxic to recipients at the dosages and concentrations employed and is

5 compatible with other ingredients of the formulation. For example, the formulation preferably does not include oxidizing agents and other compounds that are known to be deleterious to the Therapeutic.

10 Generally, the formulations are prepared by contacting the Therapeutic uniformly and intimately with liquid carriers or finely divided solid carriers or both. Then, if necessary, the product is shaped into the desired formulation. Preferably the carrier is a parenteral carrier, more preferably a solution that is isotonic with the blood of the recipient. Examples of such carrier vehicles include water, saline, Ringer's solution, and dextrose solution. Non-aqueous vehicles such as fixed oils and ethyl oleate are also useful herein, as well as liposomes.

15 The carrier suitably contains minor amounts of additives such as substances that enhance isotonicity and chemical stability. Such materials are non-toxic to recipients at the dosages and concentrations employed, and include buffers such as phosphate, citrate, succinate, acetic acid, and other organic acids or their salts; antioxidants such as ascorbic acid; low molecular weight (less than about ten residues) polypeptides, e.g., polyarginine 20 or tripeptides; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids, such as glycine, glutamic acid, aspartic acid, or arginine; monosaccharides, disaccharides, and other carbohydrates including cellulose or its derivatives, glucose, mannose, or dextrins; chelating agents such 25 as EDTA; sugar alcohols such as mannitol or sorbitol; counterions such as sodium; and/or nonionic surfactants such as polysorbates, poloxamers, or PEG.

30 The Therapeutic will typically be formulated in such vehicles at a concentration of about 0.1 mg/ml to 100 mg/ml, preferably 1-10 mg/ml, at a pH of about 3 to 8. It will be understood that the use of certain of the foregoing excipients, carriers, or stabilizers 30 will result in the formation of polypeptide salts.

35 Any pharmaceutical used for therapeutic administration can be sterile. Sterility is readily accomplished by filtration through sterile filtration membranes (e.g., 0.2 micron membranes). Therapeutics generally are placed into a container having a sterile access port, for example, an intravenous solution bag or vial having a stopper pierceable by a hypodermic injection needle.

Therapeutics ordinarily will be stored in unit or multi-dose containers, for example, sealed ampoules or vials, as an aqueous solution or as a lyophilized formulation

for reconstitution. As an example of a lyophilized formulation, 10-ml vials are filled with 5 5 ml of sterile-filtered 1% (w/v) aqueous Therapeutic solution, and the resulting mixture is lyophilized. The infusion solution is prepared by reconstituting the lyophilized Therapeutic using bacteriostatic Water-for-Injection.

The invention also provides a pharmaceutical pack or kit comprising one or more 10 containers filled with one or more of the ingredients of the Therapeutics of the invention. Associated with such container(s) can be a notice in the form prescribed by a governmental agency regulating the manufacture, use or sale of pharmaceuticals or 15 biological products, which notice reflects approval by the agency of manufacture, use or sale for human administration. In addition, the Therapeutics may be employed in conjunction with other therapeutic compounds.

The Therapeutics of the invention may be administered alone or in combination with adjuvants. Adjuvants that may be administered with the Therapeutics of the invention include, but are not limited to, alum, alum plus deoxycholate (ImmunoAg), 20 MTP-PE (Biocine Corp.), QS21 (Genentech, Inc.), BCG, and MPL. In a specific embodiment, Therapeutics of the invention are administered in combination with alum. In another specific embodiment, Therapeutics of the invention are administered in combination with QS-21. Further adjuvants that may be administered with the 25 Therapeutics of the invention include, but are not limited to, Monophosphoryl lipid immunomodulator, AdjuVax 100a, QS-21, QS-18, CRL1005, Aluminum salts, MF-59, and Virosomal adjuvant technology. Vaccines that may be administered with the Therapeutics of the invention include, but are not limited to, vaccines directed toward 30 protection against MMR (measles, mumps, rubella), polio, varicella, tetanus/diphtheria, hepatitis A, hepatitis B, haemophilus influenzae B, whooping cough, pneumonia, influenza, Lyme's Disease, rotavirus, cholera, yellow fever, Japanese encephalitis, poliomyelitis, rabies, typhoid fever, and pertussis. Combinations may be administered either concomitantly, e.g., as an admixture, separately but simultaneously or concurrently; 35 or sequentially. This includes presentations in which the combined agents are administered together as a therapeutic mixture, and also procedures in which the combined agents are administered separately but simultaneously, e.g., as through separate intravenous lines into the same individual. Administration "in combination" further includes the separate administration of one of the compounds or agents given first,

followed by the second.

5 The Therapeutics of the invention may be administered alone or in combination with other therapeutic agents. Therapeutic agents that may be administered in combination with the Therapeutics of the invention, include but not limited to, other members of the TNF family, chemotherapeutic agents, antibiotics, steroid and non-steroidal anti-inflammatories, conventional immunotherapeutic agents, cytokines and/or 10 growth factors. Combinations may be administered either concomitantly, e.g., as an admixture, separately but simultaneously or concurrently; or sequentially. This includes presentations in which the combined agents are administered together as a therapeutic mixture, and also procedures in which the combined agents are administered separately 15 but simultaneously, e.g., as through separate intravenous lines into the same individual. Administration "in combination" further includes the separate administration of one of the compounds or agents given first, followed by the second.

In one embodiment, the Therapeutics of the invention are administered in combination with members of the TNF family. TNF, TNF-related or TNF-like molecules 20 that may be administered with the Therapeutics of the invention include, but are not limited to, soluble forms of TNF-alpha, lymphotoxin-alpha (LT-alpha, also known as TNF-beta), LT-beta (found in complex heterotrimer LT-alpha2-beta), OPGL, FasL, CD27L, CD30L, CD40L, 4-1BBL, DcR3, OX40L, TNF-gamma (International 25 Publication No. WO 96/14328), AIM-I (International Publication No. WO 97/33899), endokine-alpha (International Publication No. WO 98/07880), TR6 (International Publication No. WO 98/30694), OPG, and neutrokine-alpha (International Publication No. WO 98/18921, OX40, and nerve growth factor (NGF), and soluble forms of Fas, 30 CD30, CD27, CD40 and 4-IBB, TR2 (International Publication No. WO 96/34095), DR3 (International Publication No. WO 97/33904), DR4 (International Publication No. WO 98/32856), TR5 (International Publication No. WO 98/30693), TR6 (International Publication No. WO 98/30694), TR7 (International Publication No. WO 98/41629), TRANK, TR9 (International Publication No. WO 98/56892), TR10 (International 35 Publication No. WO 98/54202), 312C2 (International Publication No. WO 98/06842), and TR12, and soluble forms CD154, CD70, and CD153.

In certain embodiments, Therapeutics of the invention are administered in combination with antiretroviral agents, nucleoside reverse transcriptase inhibitors, non-

nucleoside reverse transcriptase inhibitors, and/or protease inhibitors. Nucleoside reverse  
5 transcriptase inhibitors that may be administered in combination with the Therapeutics  
of the invention, include, but are not limited to, RETROVIR (zidovudine/AZT), VIDEX  
(didanosine/ddI), HIVID (zalcitabine/ddC), ZERIT (stavudine/d4T), EPIVIR  
(lamivudine/3TC), and COMBIVIR (zidovudine/lamivudine). Non-nucleoside reverse  
10 transcriptase inhibitors that may be administered in combination with the Therapeutics  
of the invention, include, but are not limited to, VIRAMUNE (nevirapine),  
RESCRIPTOR (delavirdine), and SUSTIVA (efavirenz). Protease inhibitors that may be  
administered in combination with the Therapeutics of the invention, include, but are not  
15 limited to, CRIXIVAN (indinavir), NORVIR (ritonavir), INVIRASE (saquinavir), and  
VIRACEPT (nelfinavir). In a specific embodiment, antiretroviral agents, nucleoside  
reverse transcriptase inhibitors, non-nucleoside reverse transcriptase inhibitors, and/or  
protease inhibitors may be used in any combination with Therapeutics of the invention  
to treat AIDS and/or to prevent or treat HIV infection.

In other embodiments, Therapeutics of the invention may be administered in  
20 combination with anti-opportunistic infection agents. Anti-opportunistic agents that may  
be administered in combination with the Therapeutics of the invention, include, but are  
not limited to, TRIMETHOPRIM-SULFAMETHOXAZOLE, DAPSONE,  
PENTAMIDINE, ATOVAQUONE, ISONIAZID, RIFAMPIN, PYRAZINAMIDE,  
25 ETHAMBUTOL, RIFABUTIN, CLARITHROMYCIN, AZITHROMYCIN,  
GANCICLOVIR, FOSCARNET, CIDOFOVIR, FLUCONAZOLE, ITRACONAZOLE,  
KETOCONAZOLE, ACYCLOVIR, FAMCICOLVIR, PYRIMETHAMINE,  
LEUCOVORIN, NEUPOGEN (filgrastim/G-CSF), and LEUKINE (sargramostim/GM-  
CSF). In a specific embodiment, Therapeutics of the invention are used in any  
30 combination with TRIMETHOPRIM-SULFAMETHOXAZOLE, DAPSONE,  
PENTAMIDINE, and/or ATOVAQUONE to prophylactically treat or prevent an  
opportunistic *Pneumocystis carinii* pneumonia infection. In another specific embodiment,  
Therapeutics of the invention are used in any combination with ISONIAZID, RIFAMPIN,  
35 PYRAZINAMIDE, and/or ETHAMBUTOL to prophylactically treat or prevent an  
opportunistic *Mycobacterium avium* complex infection. In another specific embodiment,  
Therapeutics of the invention are used in any combination with RIFABUTIN,  
CLARITHROMYCIN, and/or AZITHROMYCIN to prophylactically treat or prevent an

opportunistic *Mycobacterium tuberculosis* infection. In another specific embodiment,

5 Therapeutics of the invention are used in any combination with **GANCICLOVIR**, **FOSCARNET**, and/or **CIDOFOVIR** to prophylactically treat or prevent an opportunistic cytomegalovirus infection. In another specific embodiment, Therapeutics of the invention are used in any combination with **FLUCONAZOLE**, **ITRACONAZOLE**, and/or **KETOCONAZOLE** to prophylactically treat or prevent an opportunistic fungal infection.

10 In another specific embodiment, Therapeutics of the invention are used in any combination with **ACYCLOVIR** and/or **FAMCICOLVIR** to prophylactically treat or prevent an opportunistic herpes simplex virus type I and/or type II infection. In another specific embodiment, Therapeutics of the invention are used in any combination with

15 **PYRIMETHAMINE** and/or **LEUCOVORIN** to prophylactically treat or prevent an opportunistic *Toxoplasma gondii* infection. In another specific embodiment, Therapeutics of the invention are used in any combination with **LEUCOVORIN** and/or **NEUPOGEN** to prophylactically treat or prevent an opportunistic bacterial infection.

In a further embodiment, the Therapeutics of the invention are administered in

20 combination with an antiviral agent. Antiviral agents that may be administered with the Therapeutics of the invention include, but are not limited to, acyclovir, ribavirin, amantadine, and remantidine.

In a further embodiment, the Therapeutics of the invention are administered in

25 combination with an antibiotic agent. Antibiotic agents that may be administered with the Therapeutics of the invention include, but are not limited to, amoxicillin, beta-lactamases, aminoglycosides, beta-lactam (glycopeptide), beta-lactamases, Clindamycin, chloramphenicol, cephalosporins, ciprofloxacin, ciprofloxacin, erythromycin, fluoroquinolones, macrolides, metronidazole, penicillins, quinolones, rifampin,

30 streptomycin, sulfonamide, tetracyclines, trimethoprim, trimethoprim-sulfamthoxazole, and vancomycin.

Conventional nonspecific immunosuppressive agents, that may be administered in combination with the Therapeutics of the invention include, but are not limited to, steroids, cyclosporine, cyclosporine analogs, cyclophosphamide methylprednisolone, prednisone, azathioprine, FK-506, 15-deoxyspergualin, and other immunosuppressive agents that act by suppressing the function of responding T cells.

In specific embodiments, Therapeutics of the invention are administered in

combination with immunosuppressants. Immunosuppressants preparations that may be  
5 administered with the Therapeutics of the invention include, but are not limited to,  
ORTHOCLONE (OKT3), SANDIMMUNE/NEORAL/SANGDYA (cyclosporin),  
PROGRAF (tacrolimus), CELLCEPT (mycophenolate), Azathioprine, glucorticosteroids,  
and RAPAMUNE (sirolimus). In a specific embodiment, immunosuppressants may be  
used to prevent rejection of organ or bone marrow transplantation.

10 In an additional embodiment, Therapeutics of the invention are administered  
alone or in combination with one or more intravenous immune globulin preparations.  
Intravenous immune globulin preparations that may be administered with the  
Therapeutics of the invention include, but not limited to, GAMMAR, IVEEGAM,  
15 SANDOGLOBULIN, GAMMAGARD S/D, and GAMIMUNE. In a specific  
embodiment, Therapeutics of the invention are administered in combination with  
intravenous immune globulin preparations in transplantation therapy (e.g., bone marrow  
transplant).

20 In an additional embodiment, the Therapeutics of the invention are administered  
alone or in combination with an anti-inflammatory agent. Anti-inflammatory agents that  
may be administered with the Therapeutics of the invention include, but are not limited  
to, glucocorticoids and the nonsteroidal anti-inflammatories, aminoarylcarboxylic acid  
derivatives, arylacetic acid derivatives, arylbutyric acid derivatives, arylcarboxylic acids,  
25 arylpropionic acid derivatives, pyrazoles, pyrazolones, salicylic acid derivatives,  
thiazinecarboxamides,  $\epsilon$ -acetamidocaproic acid, S-adenosylmethionine, 3-amino-4-  
hydroxybutyric acid, amixetrine, bendazac, benzydamine, bucolome, difenpiramide,  
ditazol, emorfazole, guiazulene, nabumetone, nimesulide, orgotein, oxaceprol,  
paranyline, perisoxal, pifoxime, proquazone, proxazole, and tenidap.

30 In another embodiment, compositions of the invention are administered in  
combination with a chemotherapeutic agent. Chemotherapeutic agents that may be  
administered with the Therapeutics of the invention include, but are not limited to,  
antibiotic derivatives (e.g., doxorubicin, bleomycin, daunorubicin, and dactinomycin);  
35 antiestrogens (e.g., tamoxifen); antimetabolites (e.g., fluorouracil, 5-FU, methotrexate,  
flouxuridine, interferon alpha-2b, glutamic acid, plicamycin, mercaptourine, and 6-  
thioguanine); cytotoxic agents (e.g., carmustine, BCNU, lomustine, CCNU, cytosine  
arabinoside, cyclophosphamide, estramustine, hydroxyurea, procarbazine, mitomycin,

busulfan, cis-platin, and vincristine sulfate); hormones (e.g., medroxyprogesterone, 5 estramustine phosphate sodium, ethinyl estradiol, estradiol, megestrol acetate, methyltestosterone, diethylstilbestrol diphosphate, chlorotrianisene, and testolactone); nitrogen mustard derivatives (e.g., mephalen, chorambucil, mechlorethamine (nitrogen mustard) and thiotepa); steroids and combinations (e.g., bethamethasone sodium phosphate); and others (e.g., dacarbazine, asparaginase, mitotane, vincristine sulfate, 10 vinblastine sulfate, and etoposide).

In a specific embodiment, Therapeutics of the invention are administered in combination with CHOP (cyclophosphamide, doxorubicin, vincristine, and prednisone) or any combination of the components of CHOP. In another embodiment, Therapeutics 15 of the invention are administered in combination with Rituximab. In a further embodiment, Therapeutics of the invention are administered with Rituximab and CHOP, or Rituximab and any combination of the components of CHOP.

In an additional embodiment, the Therapeutics of the invention are administered 20 in combination with cytokines. Cytokines that may be administered with the Therapeutics of the invention include, but are not limited to, IL2, IL3, IL4, IL5, IL6, IL7, IL10, IL12, IL13, IL15, anti-CD40, CD40L, IFN-gamma and TNF-alpha. In another embodiment, Therapeutics of the invention may be administered with any interleukin, including, but not limited to, IL-1alpha, IL-1beta, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, 25 IL-11, IL-12, IL-13, IL-14, IL-15, IL-16, IL-17, IL-18, IL-19, IL-20, and IL-21.

In an additional embodiment, the Therapeutics of the invention are administered 30 in combination with angiogenic proteins. Angiogenic proteins that may be administered with the Therapeutics of the invention include, but are not limited to, Glioma Derived Growth Factor (GDGF), as disclosed in European Patent Number EP-399816; Platelet Derived Growth Factor-A (PDGF-A), as disclosed in European Patent Number EP-682110; Platelet Derived Growth Factor-B (PDGF-B), as disclosed in European Patent Number EP-282317; Placental Growth Factor (PIGF), as disclosed in International Publication Number WO 92/06194; Placental Growth Factor-2 (PIGF-2), as disclosed in Hauser et al., Gorwth Factors, 4:259-268 (1993); Vascular Endothelial Growth Factor 35 (VEGF), as disclosed in International Publication Number WO 90/13649; Vascular Endothelial Growth Factor-A (VEGF-A), as disclosed in European Patent Number EP-506477; Vascular Endothelial Growth Factor-2 (VEGF-2), as disclosed in International

Publication Number WO 96/39515; Vascular Endothelial Growth Factor B (VEGF-3);  
5 Vascular Endothelial Growth Factor B-186 (VEGF-B186), as disclosed in International Publication Number WO 96/26736; Vascular Endothelial Growth Factor-D (VEGF-D), as disclosed in International Publication Number WO 98/02543; Vascular Endothelial Growth Factor-D (VEGF-D), as disclosed in International Publication Number WO 98/07832; and Vascular Endothelial Growth Factor-E (VEGF-E), as disclosed in German 10 Patent Number DE19639601. The above mentioned references are incorporated herein by reference herein.

In an additional embodiment, the Therapeutics of the invention are administered in combination with hematopoietic growth factors. Hematopoietic growth factors that 15 may be administered with the Therapeutics of the invention include, but are not limited to, LEUKINE (SARGRAMOSTIM) and NEUPOGEN (FILGRASTIM).

In an additional embodiment, the Therapeutics of the invention are administered in combination with Fibroblast Growth Factors. Fibroblast Growth Factors that may be administered with the Therapeutics of the invention include, but are not limited to, FGF-20 1, FGF-2, FGF-3, FGF-4, FGF-5, FGF-6, FGF-7, FGF-8, FGF-9, FGF-10, FGF-11, FGF-12, FGF-13, FGF-14, and FGF-15.

In a specific embodiment, formulations of the present invention may further comprise antagonists of P-glycoprotein (also referred to as the multi-resistance protein, or PGP), including antagonists of its encoding polynucleotides (e.g., antisense 25 oligonucleotides, ribozymes, zinc-finger proteins, etc.). P-glycoprotein is well known for decreasing the efficacy of various drug administrations due to its ability to export intracellular levels of absorbed drug to the cell exterior. While this activity has been particularly pronounced in cancer cells in response to the administration of chemotherapy 30 regimens, a variety of other cell types and the administration of other drug classes have been noted (e.g., T-cells and anti-HIV drugs). In fact, certain mutations in the PGP gene significantly reduces PGP function, making it less able to force drugs out of cells. People who have two versions of the mutated gene--one inherited from each parent--have more 35 than four times less PGP than those with two normal versions of the gene. People may also have one normal gene and one mutated one. Certain ethnic populations have increased incidence of such PGP mutations. Among individuals from Ghana, Kenya, the Sudan, as well as African Americans, frequency of the normal gene ranged from 73% to

84%. In contrast, the frequency was 34% to 59% among British whites, Portuguese, 5 Southwest Asian, Chinese, Filipino and Saudi populations. As a result, certain ethnic populations may require increased administration of PGP antagonist in the formulation of the present invention to arrive at the an efficacious dose of the therapeutic (e.g., those from african descent). Conversely, certain ethnic populations, particularly those having increased frequency of the mutated PGP (e.g., of caucasian descent, or non-african 10 descent) may require less pharmaceutical compositions in the formulation due to an effective increase in efficacy of such compositions as a result of the increased effective absorption (e.g., less PGP activity) of said composition.

In additional embodiments, the Therapeutics of the invention are administered in 15 combination with other therapeutic or prophylactic regimens, such as, for example, radiation therapy.

**Example 33 - Method Of Treating Decreased Levels Of The Polypeptide.**

The present invention relates to a method for treating an individual in need of an 20 increased level of a polypeptide of the invention in the body comprising administering to such an individual a composition comprising a therapeutically effective amount of an agonist of the invention (including polypeptides of the invention). Moreover, it will be appreciated that conditions caused by a decrease in the standard or normal expression 25 level of a secreted protein in an individual can be treated by administering the polypeptide of the present invention, preferably in the secreted form. Thus, the invention also provides a method of treatment of an individual in need of an increased level of the polypeptide comprising administering to such an individual a Therapeutic comprising an amount of the polypeptide to increase the activity level of the polypeptide in such an 30 individual.

For example, a patient with decreased levels of a polypeptide receives a daily dose 35 0.1-100 ug/kg of the polypeptide for six consecutive days. Preferably, the polypeptide is in the secreted form. The exact details of the dosing scheme, based on administration and formulation, are provided herein.

**Example 34 - Method Of Treating Increased Levels Of The Polypeptide.**

The present invention also relates to a method of treating an individual in need

of a decreased level of a polypeptide of the invention in the body comprising  
5 administering to such an individual a composition comprising a therapeutically effective  
amount of an antagonist of the invention (including polypeptides and antibodies of the  
invention).

In one example, antisense technology is used to inhibit production of a  
10 polypeptide of the present invention. This technology is one example of a method of  
decreasing levels of a polypeptide, preferably a secreted form, due to a variety of  
etiologies, such as cancer. For example, a patient diagnosed with abnormally increased  
levels of a polypeptide is administered intravenously antisense polynucleotides at 0.5,  
1.0, 1.5, 2.0 and 3.0 mg/kg day for 21 days. This treatment is repeated after a 7-day rest  
15 period if the treatment was well tolerated. The formulation of the antisense  
polynucleotide is provided herein.

**Example 35 - Method Of Treatment Using Gene Therapy-Ex Vivo.**

One method of gene therapy transplants fibroblasts, which are capable of  
20 expressing a polypeptide, onto a patient. Generally, fibroblasts are obtained from a  
subject by skin biopsy. The resulting tissue is placed in tissue-culture medium and  
separated into small pieces. Small chunks of the tissue are placed on a wet surface of a  
tissue culture flask, approximately ten pieces are placed in each flask. The flask is turned  
25 upside down, closed tight and left at room temperature over night. After 24 hours at room  
temperature, the flask is inverted and the chunks of tissue remain fixed to the bottom of  
the flask and fresh media (e.g., Ham's F12 media, with 10% FBS, penicillin and  
streptomycin) is added. The flasks are then incubated at 37 degree C for approximately  
one week.

30 At this time, fresh media is added and subsequently changed every several days.  
After an additional two weeks in culture, a monolayer of fibroblasts emerge. The  
monolayer is trypsinized and scaled into larger flasks.

35 pMV-7 (Kirschmeier, P.T. et al., DNA, 7:219-25 (1988)), flanked by the long  
terminal repeats of the Moloney murine sarcoma virus, is digested with EcoRI and  
HindIII and subsequently treated with calf intestinal phosphatase. The linear vector is  
fractionated on agarose gel and purified, using glass beads.

The cDNA encoding a polypeptide of the present invention can be amplified using

PCR primers which correspond to the 5' and 3' end sequences respectively as set forth in  
5 Example 20 using primers and having appropriate restriction sites and initiation/stop codons, if necessary. Preferably, the 5' primer contains an EcoRI site and the 3' primer includes a HindIII site. Equal quantities of the Moloney murine sarcoma virus linear backbone and the amplified EcoRI and HindIII fragment are added together, in the presence of T4 DNA ligase. The resulting mixture is maintained under conditions  
10 appropriate for ligation of the two fragments. The ligation mixture is then used to transform bacteria HB101, which are then plated onto agar containing kanamycin for the purpose of confirming that the vector has the gene of interest properly inserted.

The amphotropic pA317 or GP+am12 packaging cells are grown in tissue culture  
15 to confluent density in Dulbecco's Modified Eagles Medium (DMEM) with 10% calf serum (CS), penicillin and streptomycin. The MSV vector containing the gene is then added to the media and the packaging cells transduced with the vector. The packaging cells now produce infectious viral particles containing the gene (the packaging cells are now referred to as producer cells).

20 Fresh media is added to the transduced producer cells, and subsequently, the media is harvested from a 10 cm plate of confluent producer cells. The spent media, containing the infectious viral particles, is filtered through a millipore filter to remove detached producer cells and this media is then used to infect fibroblast cells. Media is  
25 removed from a sub-confluent plate of fibroblasts and quickly replaced with the media from the producer cells. This media is removed and replaced with fresh media. If the titer of virus is high, then virtually all fibroblasts will be infected and no selection is required. If the titer is very low, then it is necessary to use a retroviral vector that has a selectable marker, such as neo or his. Once the fibroblasts have been efficiently infected, the  
30 fibroblasts are analyzed to determine whether protein is produced.

The engineered fibroblasts are then transplanted onto the host, either alone or after having been grown to confluence on cytodex 3 microcarrier beads.

35 **Example 36 - Gene Therapy Using Endogenous Genes Corresponding To Polynucleotides Of The Invention.**

Another method of gene therapy according to the present invention involves operably associating the endogenous polynucleotide sequence of the invention with a

promoter via homologous recombination as described, for example, in U.S. Patent NO: 5,641,670, issued June 24, 1997; International Publication NO: WO 96/29411, published September 26, 1996; International Publication NO: WO 94/12650, published August 4, 1994; Koller et al., Proc. Natl. Acad. Sci. USA, 86:8932-8935 (1989); and Zijlstra et al., Nature, 342:435-438 (1989). This method involves the activation of a gene which is present in the target cells, but which is not expressed in the cells, or is expressed at a lower level than desired.

10 Polynucleotide constructs are made which contain a promoter and targeting sequences, which are homologous to the 5' non-coding sequence of endogenous polynucleotide sequence, flanking the promoter. The targeting sequence will be sufficiently near the 5' end of the polynucleotide sequence so the promoter will be operably linked to the endogenous sequence upon homologous recombination. The promoter and the targeting sequences can be amplified using PCR. Preferably, the amplified promoter contains distinct restriction enzyme sites on the 5' and 3' ends. Preferably, the 3' end of the first targeting sequence contains the same restriction enzyme 15 site as the 5' end of the amplified promoter and the 5' end of the second targeting sequence contains the same restriction site as the 3' end of the amplified promoter.

20 The amplified promoter and the amplified targeting sequences are digested with the appropriate restriction enzymes and subsequently treated with calf intestinal phosphatase. The digested promoter and digested targeting sequences are added together 25 in the presence of T4 DNA ligase. The resulting mixture is maintained under conditions appropriate for ligation of the two fragments. The construct is size fractionated on an agarose gel then purified by phenol extraction and ethanol precipitation.

25 In this Example, the polynucleotide constructs are administered as naked 30 polynucleotides via electroporation. However, the polynucleotide constructs may also be administered with transfection-facilitating agents, such as liposomes, viral sequences, viral particles, precipitating agents, etc. Such methods of delivery are known in the art.

35 Once the cells are transfected, homologous recombination will take place which results in the promoter being operably linked to the endogenous polynucleotide sequence. This results in the expression of polynucleotide corresponding to the polynucleotide in the cell. Expression may be detected by immunological staining, or any other method known in the art.

5 Fibroblasts are obtained from a subject by skin biopsy. The resulting tissue is placed in DMEM + 10% fetal calf serum. Exponentially growing or early stationary phase fibroblasts are trypsinized and rinsed from the plastic surface with nutrient medium. An aliquot of the cell suspension is removed for counting, and the remaining cells are subjected to centrifugation. The supernatant is aspirated and the pellet is resuspended in 10 5 ml of electroporation buffer (20 mM HEPES pH 7.3, 137 mM NaCl, 5 mM KCl, 0.7 mM Na<sub>2</sub> HPO<sub>4</sub>, 6 mM dextrose). The cells are recentrifuged, the supernatant aspirated, and the cells resuspended in electroporation buffer containing 1 mg/ml acetylated bovine serum albumin. The final cell suspension contains approximately 3X10<sup>6</sup> cells/ml. Electroporation should be performed immediately following resuspension.

15 10 Plasmid DNA is prepared according to standard techniques. For example, to construct a plasmid for targeting to the locus corresponding to the polynucleotide of the invention, plasmid pUC18 (MBI Fermentas, Amherst, NY) is digested with HindIII. The CMV promoter is amplified by PCR with an XbaI site on the 5' end and a BamHI site on the 3'end. Two non-coding sequences are amplified via PCR: one non-coding sequence 20 (fragment 1) is amplified with a HindIII site at the 5'end and an Xba site at the 3'end; the other non-coding sequence (fragment 2) is amplified with a BamHI site at the 5'end and a HindIII site at the 3'end. The CMV promoter and the fragments (1 and 2) are digested with the appropriate enzymes (CMV promoter - XbaI and BamHI; fragment 1 - XbaI; 25 fragment 2 - BamHI) and ligated together. The resulting ligation product is digested with HindIII, and ligated with the HindIII-digested pUC18 plasmid.

Plasmid DNA is added to a sterile cuvette with a 0.4 cm electrode gap (Bio-Rad). The final DNA concentration is generally at least 120 µg/ml. 0.5 ml of the cell suspension (containing approximately 1.5.X10<sup>6</sup> cells) is then added to the cuvette, and 30 the cell suspension and DNA solutions are gently mixed. Electroporation is performed with a Gene-Pulser apparatus (Bio-Rad). Capacitance and voltage are set at 960 µF and 250-300 V, respectively. As voltage increases, cell survival decreases, but the percentage of surviving cells that stably incorporate the introduced DNA into their genome increases dramatically. Given these parameters, a pulse time of approximately 14-20 mSec should 35 be observed.

Electroporated cells are maintained at room temperature for approximately 5 min, and the contents of the cuvette are then gently removed with a sterile transfer pipette. The

5 cells are added directly to 10 ml of prewarmed nutrient media (DMEM with 15% calf serum) in a 10 cm dish and incubated at 37 degree C. The following day, the media is aspirated and replaced with 10 ml of fresh media and incubated for a further 16-24 hours.

10 The engineered fibroblasts are then injected into the host, either alone or after having been grown to confluence on cytodex 3 microcarrier beads. The fibroblasts now produce the protein product. The fibroblasts can then be introduced into a patient as described above.

**Example 37 - Method Of Treatment Using Gene Therapy - In Vivo.**

15 Another aspect of the present invention is using in vivo gene therapy methods to treat disorders, diseases and conditions. The gene therapy method relates to the introduction of naked nucleic acid (DNA, RNA, and antisense DNA or RNA) sequences into an animal to increase or decrease the expression of the polypeptide. The polynucleotide of the present invention may be operatively linked to a promoter or any other genetic elements necessary for the expression of the polypeptide by the target tissue.

20 Such gene therapy and delivery techniques and methods are known in the art, see, for example, WO90/11092, WO98/11779; U.S. Patent NO. 5693622, 5705151, 5580859; Tabata et al., *Cardiovasc. Res.* 35(3):470-479 (1997); Chao et al., *Pharmacol. Res.* 35(6):517-522 (1997); Wolff, *Neuromuscul. Disord.* 7(5):314-318 (1997); Schwartz et al., *Gene Ther.* 3(5):405-411 (1996); Tsurumi et al., *Circulation* 94(12):3281-3290 (1996) 25 (incorporated herein by reference).

30 The polynucleotide constructs may be delivered by any method that delivers injectable materials to the cells of an animal, such as, injection into the interstitial space of tissues (heart, muscle, skin, lung, liver, intestine and the like). The polynucleotide constructs can be delivered in a pharmaceutically acceptable liquid or aqueous carrier.

35 The term "naked" polynucleotide, DNA or RNA, refers to sequences that are free from any delivery vehicle that acts to assist, promote, or facilitate entry into the cell, including viral sequences, viral particles, liposome formulations, lipofectin or precipitating agents and the like. However, the polynucleotides of the present invention may also be delivered in liposome formulations (such as those taught in Felgner P.L. et al. (1995) *Ann. NY Acad. Sci.* 772:126-139 and Abdallah B. et al. (1995) *Biol. Cell* 85(1):1-7) which can be prepared by methods well known to those skilled in the art.

The polynucleotide vector constructs used in the gene therapy method are 5 preferably constructs that will not integrate into the host genome nor will they contain sequences that allow for replication. Any strong promoter known to those skilled in the art can be used for driving the expression of DNA. Unlike other gene therapies techniques, one major advantage of introducing naked nucleic acid sequences into target 10 cells is the transitory nature of the polynucleotide synthesis in the cells. Studies have shown that non-replicating DNA sequences can be introduced into cells to provide production of the desired polypeptide for periods of up to six months.

The polynucleotide construct can be delivered to the interstitial space of tissues within the an animal, including of muscle, skin, brain, lung, liver, spleen, bone marrow, 15 thymus, heart, lymph, blood, bone, cartilage, pancreas, kidney, gall bladder, stomach, intestine, testis, ovary, uterus, rectum, nervous system, eye, gland, and connective tissue. Interstitial space of the tissues comprises the intercellular fluid, mucopolysaccharide matrix among the reticular fibers of organ tissues, elastic fibers in the walls of vessels or 20 chambers, collagen fibers of fibrous tissues, or that same matrix within connective tissue ensheathing muscle cells or in the lacunae of bone. It is similarly the space occupied by the plasma of the circulation and the lymph fluid of the lymphatic channels. Delivery to 25 the interstitial space of muscle tissue is preferred for the reasons discussed below. They may be conveniently delivered by injection into the tissues comprising these cells. They are preferably delivered to and expressed in persistent, non-dividing cells which are differentiated, although delivery and expression may be achieved in non-differentiated or less completely differentiated cells, such as, for example, stem cells of blood or skin fibroblasts. In vivo muscle cells are particularly competent in their ability to take up and express polynucleotides.

30 For the naked polynucleotide injection, an effective dosage amount of DNA or RNA will be in the range of from about 0.05 g/kg body weight to about 50 mg/kg body weight. Preferably the dosage will be from about 0.005 mg/kg to about 20 mg/kg and more preferably from about 0.05 mg/kg to about 5 mg/kg. Of course, as the artisan of 35 ordinary skill will appreciate, this dosage will vary according to the tissue site of injection. The appropriate and effective dosage of nucleic acid sequence can readily be determined by those of ordinary skill in the art and may depend on the condition being treated and the route of administration. The preferred route of administration is by the

parenteral route of injection into the interstitial space of tissues. However, other 5 parenteral routes may also be used, such as, inhalation of an aerosol formulation particularly for delivery to lungs or bronchial tissues, throat or mucous membranes of the nose. In addition, naked polynucleotide constructs can be delivered to arteries during angioplasty by the catheter used in the procedure.

10 The dose response effects of injected polynucleotide in muscle in vivo is determined as follows. Suitable template DNA for production of mRNA coding for polypeptide of the present invention is prepared in accordance with a standard recombinant DNA methodology. The template DNA, which may be either circular or linear, is either used as naked DNA or complexed with liposomes. The quadriceps 15 muscles of mice are then injected with various amounts of the template DNA.

Five to six week old female and male Balb/C mice are anesthetized by 20 intraperitoneal injection with 0.3 ml of 2.5% Avertin. A 1.5 cm incision is made on the anterior thigh, and the quadriceps muscle is directly visualized. The template DNA is injected in 0.1 ml of carrier in a 1 cc syringe through a 27 gauge needle over one minute, 25 approximately 0.5 cm from the distal insertion site of the muscle into the knee and about 0.2 cm deep. A suture is placed over the injection site for future localization, and the skin is closed with stainless steel clips.

After an appropriate incubation time (e.g., 7 days) muscle extracts are prepared 25 by excising the entire quadriceps. Every fifth 15 um cross-section of the individual quadriceps muscles is histochemically stained for protein expression. A time course for protein expression may be done in a similar fashion except that quadriceps from different mice are harvested at different times. Persistence of DNA in muscle following injection 30 may be determined by Southern blot analysis after preparing total cellular DNA and HIRT supernatants from injected and control mice. The results of the above experimentation in mice can be used to extrapolate proper dosages and other treatment parameters in humans and other animals using naked DNA.

**35 Example 38 - Transgenic Animals.**  
The polypeptides of the invention can also be expressed in transgenic animals. Animals of any species, including, but not limited to, mice, rats, rabbits, hamsters, guinea pigs, pigs, micro-pigs, goats, sheep, cows and non-human primates, e.g., baboons,

monkeys, and chimpanzees may be used to generate transgenic animals. In a specific 5 embodiment, techniques described herein or otherwise known in the art, are used to express polypeptides of the invention in humans, as part of a gene therapy protocol.

Any technique known in the art may be used to introduce the transgene (i.e., polynucleotides of the invention) into animals to produce the founder lines of transgenic animals. Such techniques include, but are not limited to, pronuclear microinjection 10 (Paterson et al., *Appl. Microbiol. Biotechnol.* 40:691-698 (1994); Carver et al., *Biotechnology (NY)* 11:1263-1270 (1993); Wright et al., *Biotechnology (NY)* 9:830-834 (1991); and Hoppe et al., U.S. Pat. No. 4,873,191 (1989)); retrovirus mediated gene transfer into germ lines (Van der Putten et al., *Proc. Natl. Acad. Sci., USA* 82:6148-6152 15 (1985)), blastocysts or embryos; gene targeting in embryonic stem cells (Thompson et al., *Cell* 56:313-321 (1989)); electroporation of cells or embryos (Lo, 1983, *Mol Cell. Biol.* 3:1803-1814 (1983)); introduction of the polynucleotides of the invention using a gene gun (see, e.g., Ulmer et al., *Science* 259:1745 (1993); introducing nucleic acid constructs 20 into embryonic pluripotent stem cells and transferring the stem cells back into the blastocyst; and sperm-mediated gene transfer (Lavitrano et al., *Cell* 57:717-723 (1989); etc. For a review of such techniques, see Gordon, "Transgenic Animals" *Intl. Rev. Cytol.* 25 115:171-229 (1989), which is incorporated by reference herein in its entirety.

Any technique known in the art may be used to produce transgenic clones 25 containing polynucleotides of the invention, for example, nuclear transfer into enucleated oocytes of nuclei from cultured embryonic, fetal, or adult cells induced to quiescence (Campell et al., *Nature* 380:64-66 (1996); Wilmut et al., *Nature* 385:810-813 (1997)).

The present invention provides for transgenic animals that carry the transgene in all their cells, as well as animals which carry the transgene in some, but not all their cells, 30 i.e., mosaic animals or chimeric. The transgene may be integrated as a single transgene or as multiple copies such as in concatamers, e.g., head-to-head tandems or head-to-tail tandems. The transgene may also be selectively introduced into and activated in a particular cell type by following, for example, the teaching of Lasko et al. (Lasko et al., *Proc. Natl. Acad. Sci. USA* 89:6232-6236 (1992)). The regulatory sequences required for 35 such a cell-type specific activation will depend upon the particular cell type of interest, and will be apparent to those of skill in the art. When it is desired that the polynucleotide transgene be integrated into the chromosomal site of the endogenous gene, gene targeting

is preferred. Briefly, when such a technique is to be utilized, vectors containing some 5 nucleotide sequences homologous to the endogenous gene are designed for the purpose of integrating, via homologous recombination with chromosomal sequences, into and disrupting the function of the nucleotide sequence of the endogenous gene. The transgene may also be selectively introduced into a particular cell type, thus inactivating the 10 endogenous gene in only that cell type, by following, for example, the teaching of Gu et al. (Gu et al., *Science* 265:103-106 (1994)). The regulatory sequences required for such a cell-type specific inactivation will depend upon the particular cell type of interest, and will be apparent to those of skill in the art.

Once transgenic animals have been generated, the expression of the recombinant 15 gene may be assayed utilizing standard techniques. Initial screening may be accomplished by Southern blot analysis or PCR techniques to analyze animal tissues to verify that integration of the transgene has taken place. The level of mRNA expression of the transgene in the tissues of the transgenic animals may also be assessed using techniques which include, but are not limited to, Northern blot analysis of tissue samples obtained 20 from the animal, *in situ* hybridization analysis, and reverse transcriptase-PCR(RT-PCR).. Samples of transgenic gene-expressing tissue may also be evaluated immunocytochemically or immunohistochemically using antibodies specific for the transgene product.

Once the founder animals are produced, they may be bred, inbred, outbred, or 25 crossbred to produce colonies of the particular animal. Examples of such breeding strategies include, but are not limited to: outbreeding of founder animals with more than one integration site in order to establish separate lines; inbreeding of separate lines in order to produce compound transgenics that express the transgene at higher levels 30 because of the effects of additive expression of each transgene; crossing of heterozygous transgenic animals to produce animals homozygous for a given integration site in order to both augment expression and eliminate the need for screening of animals by DNA analysis; crossing of separate homozygous lines to produce compound heterozygous or 35 homozygous lines; and breeding to place the transgene on a distinct background that is appropriate for an experimental model of interest.

Transgenic animals of the invention have uses which include, but are not limited to, animal model systems useful in elaborating the biological function of polypeptides of

the present invention, studying diseases, disorders, and/or conditions associated with  
5 aberrant expression, and in screening for compounds effective in ameliorating such  
diseases, disorders, and/or conditions.

**Example 39 - Knock-Out Animals.**

10 Endogenous gene expression can also be reduced by inactivating or "knocking out" the gene and/or its promoter using targeted homologous recombination. (E.g., see Smithies et al., *Nature* 317:230-234 (1985); Thomas & Capecchi, *Cell* 51:503-512 (1987); Thompson et al., *Cell* 5:313-321 (1989); each of which is incorporated by reference herein in its entirety). For example, a mutant, non-functional polynucleotide of  
15 the invention (or a completely unrelated DNA sequence) flanked by DNA homologous to the endogenous polynucleotide sequence (either the coding regions or regulatory regions of the gene) can be used, with or without a selectable marker and/or a negative selectable marker, to transfet cells that express polypeptides of the invention *in vivo*. In another embodiment, techniques known in the art are used to generate knockouts in cells  
20 that contain, but do not express the gene of interest. Insertion of the DNA construct, via targeted homologous recombination, results in inactivation of the targeted gene. Such approaches are particularly suited in research and agricultural fields where modifications to embryonic stem cells can be used to generate animal offspring with an inactive  
25 targeted gene (e.g., see Thomas & Capecchi 1987 and Thompson 1989, *supra*). However this approach can be routinely adapted for use in humans provided the recombinant DNA constructs are directly administered or targeted to the required site *in vivo* using appropriate viral vectors that will be apparent to those of skill in the art.

30 In further embodiments of the invention, cells that are genetically engineered to express the polypeptides of the invention, or alternatively, that are genetically engineered not to express the polypeptides of the invention (e.g., knockouts) are administered to a patient *in vivo*. Such cells may be obtained from the patient (i.e., animal, including human) or an MHC compatible donor and can include, but are not limited to fibroblasts, bone marrow cells, blood cells (e.g., lymphocytes), adipocytes, muscle cells, endothelial  
35 cells etc. The cells are genetically engineered *in vitro* using recombinant DNA techniques to introduce the coding sequence of polypeptides of the invention into the cells, or alternatively, to disrupt the coding sequence and/or endogenous regulatory sequence

associated with the polypeptides of the invention, e.g., by transduction (using viral 5 vectors, and preferably vectors that integrate the transgene into the cell genome) or transfection procedures, including, but not limited to, the use of plasmids, cosmids, YACs, naked DNA, electroporation, liposomes, etc. The coding sequence of the polypeptides of the invention can be placed under the control of a strong constitutive or 10 inducible promoter or promoter/enhancer to achieve expression, and preferably secretion, of the polypeptides of the invention. The engineered cells which express and preferably secrete the polypeptides of the invention can be introduced into the patient systemically, e.g., in the circulation, or intraperitoneally.

Alternatively, the cells can be incorporated into a matrix and implanted in the 15 body, e.g., genetically engineered fibroblasts can be implanted as part of a skin graft; genetically engineered endothelial cells can be implanted as part of a lymphatic or vascular graft. (See, for example, Anderson et al. U.S. Patent No. 5,399,349; and Mulligan & Wilson, U.S. Patent No. 5,460,959 each of which is incorporated by reference herein in its entirety).

20 When the cells to be administered are non-autologous or non-MHC compatible cells, they can be administered using well known techniques which prevent the development of a host immune response against the introduced cells. For example, the 25 cells may be introduced in an encapsulated form which, while allowing for an exchange of components with the immediate extracellular environment, does not allow the introduced cells to be recognized by the host immune system.

Transgenic and "knock-out" animals of the invention have uses which include, but are not limited to, animal model systems useful in elaborating the biological function 30 of polypeptides of the present invention, studying diseases, disorders, and/or conditions associated with aberrant expression, and in screening for compounds effective in ameliorating such diseases, disorders, and/or conditions.

#### **Example 40 - Production Of An Antibody.**

35 a) Hybridoma Technology

The antibodies of the present invention can be prepared by a variety of methods. (See, Current Protocols, Chapter 2.) As one example of such methods, cells expressing HGPRBMY1 or HGPRBMY2 are administered to an animal to induce the production of

sera containing polyclonal antibodies. In a preferred method, a preparation of 5 HGPRBMY1 or HGPRBMY2 protein is prepared and purified to render it substantially free of natural contaminants. Such a preparation is then introduced into an animal in order to produce polyclonal antisera of greater specific activity.

Monoclonal antibodies specific for protein HGPRBMY1 or HGPRBMY2 are 10 prepared using hybridoma technology. (Kohler et al., *Nature* 256:495 (1975); Kohler et al., *Eur. J. Immunol.* 6:511 (1976); Kohler et al., *Eur. J. Immunol.* 6:292 (1976); Hammerling et al., in: *Monoclonal Antibodies and T-Cell Hybridomas*, Elsevier, N.Y., pp. 563-681 (1981)). In general, an animal (preferably a mouse) is immunized with 15 HGPRBMY1 or HGPRBMY2 polypeptide or, more preferably, with a secreted HGPRBMY1 or HGPRBMY2 polypeptide-expressing cell. Such polypeptide-expressing cells are cultured in any suitable tissue culture medium, preferably in Earle's modified Eagle's medium supplemented with 10% fetal bovine serum (inactivated at about 56°C), and supplemented with about 10 g/l of nonessential amino acids, about 1,000 U/ml of penicillin, and about 100 µg/ml of streptomycin.

20 The splenocytes of such mice are extracted and fused with a suitable myeloma cell line. Any suitable myeloma cell line may be employed in accordance with the present invention; however, it is preferable to employ the parent myeloma cell line (SP2O), available from the ATCC. After fusion, the resulting hybridoma cells are selectively 25 maintained in HAT medium, and then cloned by limiting dilution as described by Wands et al. (*Gastroenterology* 80:225-232 (1981)). The hybridoma cells obtained through such a selection are then assayed to identify clones which secrete antibodies capable of binding the HGPRBMY1 or HGPRBMY2 polypeptide.

Alternatively, additional antibodies capable of binding to HGPRBMY1 or 30 HGPRBMY2 polypeptide can be produced in a two-step procedure using anti-idiotypic antibodies. Such a method makes use of the fact that antibodies are themselves antigens, and therefore, it is possible to obtain an antibody that binds to a second antibody. In accordance with this method, protein specific antibodies are used to immunize an animal, preferably a mouse. The splenocytes of such an animal are then used to produce 35 hybridoma cells, and the hybridoma cells are screened to identify clones which produce an antibody whose ability to bind to the HGPRBMY1 or HGPRBMY2 protein-specific antibody can be blocked by HGPRBMY1 or HGPRBMY2. Such antibodies comprise

5 anti-idiotypic antibodies to the HGPRBMY1 or HGPRBMY2 protein-specific antibody  
and are used to immunize an animal to induce formation of further HGPRBMY1 or  
HGPRBMY2 protein-specific antibodies.

10 For in vivo use of antibodies in humans, an antibody is "humanized". Such  
antibodies can be produced using genetic constructs derived from hybridoma cells  
producing the monoclonal antibodies described above. Methods for producing chimeric  
and humanized antibodies are known in the art and are discussed herein. (See, for review,  
15 Morrison, *Science* 229:1202 (1985); Oi et al., *BioTechniques* 4:214 (1986); Cabilly et  
al., U.S. Patent No. 4,816,567; Taniguchi et al., EP 171496; Morrison et al., EP 173494;  
Neuberger et al., WO 8601533; Robinson et al., WO 8702671; Boulianne et al., *Nature*  
15 312:643 (1984); Neuberger et al., *Nature* 314:268 (1985).)

b) Isolation Of Antibody Fragments Directed

Against HGPRBMY1 or HGPRBMY2 From A Library Of scFvs

20 Naturally occurring V-genes isolated from human PBLs are constructed into a  
library of antibody fragments which contain reactivities against HGPRBMY1 or  
HGPRBMY2 to which the donor may or may not have been exposed (see e.g., U.S.  
25 Patent 5,885,793 incorporated herein by reference in its entirety).

Rescue of the Library. A library of scFvs is constructed from the RNA of human  
PBLs as described in PCT publication WO 92/01047. To rescue phage displaying  
25 antibody fragments, approximately 109 E. coli harboring the phagemid are used to  
inoculate 50 ml of 2xTY containing 1% glucose and 100 µg/ml of ampicillin (2xTY-  
AMP-GLU) and grown to an O.D. of 0.8 with shaking. Five ml of this culture is used to  
inoculate 50 ml of 2xTY-AMP-GLU, 2 x 108 TU of delta gene 3 helper (M13 delta gene  
III, see PCT publication WO 92/01047) are added and the culture incubated at 37°C for  
30 45 minutes without shaking and then at 37°C for 45 minutes with shaking. The culture  
is centrifuged at 4000 r.p.m. for 10 min. and the pellet resuspended in 2 liters of 2xTY  
containing 100 µg/ml ampicillin and 50 µg/ml kanamycin and grown overnight. Phage  
35 are prepared as described in PCT publication WO 92/01047.

M13 delta gene III is prepared as follows: M13 delta gene III helper phage does  
35 not encode gene III protein, hence the phage(mid) displaying antibody fragments have a  
greater avidity of binding to antigen. Infectious M13 delta gene III particles are made by  
growing the helper phage in cells harboring a pUC19 derivative supplying the wild type

gene III protein during phage morphogenesis. The culture is incubated for 1 hour at 37°  
5 C without shaking and then for a further hour at 37°C with shaking. Cells are spun down  
(IEC-Centra 8,400 r.p.m. for 10 min), resuspended in 300 ml 2xTY broth containing 100  
μg ampicillin/ml and 25 μg kanamycin/ml (2xTY-AMP-KAN) and grown overnight,  
shaking at 37°C. Phage particles are purified and concentrated from the culture medium  
10 by two PEG-precipitations (Sambrook et al., 1990), resuspended in 2 ml PBS and passed  
through a 0.45 μm filter (Minisart NML; Sartorius) to give a final concentration of  
approximately 10<sup>13</sup> transducing units/ml (ampicillin-resistant clones).

Panning of the Library. Immunotubes (Nunc) are coated overnight in PBS with  
4 ml of either 100 μg/ml or 10 μg/ml of a polypeptide of the present invention. Tubes are  
15 blocked with 2% Marvel-PBS for 2 hours at 37°C and then washed 3 times in PBS.  
Approximately 10<sup>13</sup> TU of phage is applied to the tube and incubated for 30 minutes at  
room temperature tumbling on an over and under turntable and then left to stand for  
another 1.5 hours. Tubes are washed 10 times with PBS 0.1% Tween-20 and 10 times  
15 with PBS. Phage are eluted by adding 1 ml of 100 mM triethylamine and rotating 15  
minutes on an under and over turntable after which the solution is immediately  
neutralized with 0.5 ml of 1.0M Tris-HCl, pH 7.4. Phage are then used to infect 10 ml  
of mid-log E. coli TG1 by incubating eluted phage with bacteria for 30 minutes at 37°C.  
The E. coli are then plated on TYE plates containing 1% glucose and 100 μg/ml  
25 ampicillin. The resulting bacterial library is then rescued with delta gene 3 helper phage  
as described above to prepare phage for a subsequent round of selection. This process is  
then repeated for a total of 4 rounds of affinity purification with tube-washing increased  
to 20 times with PBS, 0.1% Tween-20 and 20 times with PBS for rounds 3 and 4.

Characterization of Binders. Eluted phage from the 3rd and 4th rounds of  
30 selection are used to infect E. coli HB 2151 and soluble scFv is produced (Marks, et al.,  
1991) from single colonies for assay. ELISAs are performed with microtitre plates coated  
with either 10 pg/ml of the polypeptide of the present invention in 50 mM bicarbonate  
pH 9.6. Clones positive in ELISA are further characterized by PCR fingerprinting (see,  
e.g., PCT publication WO 92/01047) and then by sequencing. These ELISA positive  
35 clones may also be further characterized by techniques known in the art, such as, for  
example, epitope mapping, binding affinity, receptor signal transduction, ability to block  
or competitively inhibit antibody/antigen binding, and competitive agonistic or

antagonistic activity.

5

**Example 41 - Assays Detecting Stimulation Or Inhibition Of B Cell Proliferation And Differentiation.**

Generation of functional humoral immune responses requires both soluble and 10 cognate signaling between B-lineage cells and their microenvironment. Signals may impart a positive stimulus that allows a B-lineage cell to continue its programmed development, or a negative stimulus that instructs the cell to arrest its current developmental pathway. To date, numerous stimulatory and inhibitory signals have been found to influence B cell responsiveness including IL-2, IL-4, IL-5, IL-6, IL-7, IL10, IL- 15 13, IL-14 and IL-15. Interestingly, these signals are by themselves weak effectors but can, in combination with various co-stimulatory proteins, induce activation, proliferation, differentiation, homing, tolerance and death among B cell populations. One of the best studied classes of B-cell co-stimulatory proteins is the TNF-superfamily. Within this family CD40, CD27, and CD30 along with their respective ligands CD154, CD70, and 20 CD153 have been found to regulate a variety of immune responses. Assays which allow for the detection and/or observation of the proliferation and differentiation of these B-cell populations and their precursors are valuable tools in determining the effects various proteins may have on these B-cell populations in terms of proliferation and 25 differentiation. Listed below are two assays designed to allow for the detection of the differentiation, proliferation, or inhibition of B-cell populations and their precursors.

**In Vitro Assay-** Purified polypeptides of the invention, or truncated forms thereof, is assessed for its ability to induce activation, proliferation, differentiation or inhibition and/or death in B-cell populations and their precursors. The activity of the polypeptides 30 of the invention on purified human tonsillar B cells, measured qualitatively over the dose range from 0.1 to 10,000 ng/mL, is assessed in a standard B-lymphocyte co-stimulation assay in which purified tonsillar B cells are cultured in the presence of either formalin-fixed *Staphylococcus aureus* Cowan I (SAC) or immobilized anti-human IgM antibody 35 as the priming agent. Second signals such as IL-2 and IL-15 synergize with SAC and IgM crosslinking to elicit B cell proliferation as measured by tritiated-thymidine incorporation. Novel synergizing agents can be readily identified using this assay. The assay involves isolating human tonsillar B cells by magnetic bead (MACS) depletion of

CD3-positive cells. The resulting cell population is greater than 95% B cells as assessed  
5 by expression of CD45R(B220).

Various dilutions of each sample are placed into individual wells of a 96-well  
plate to which are added 105 B-cells suspended in culture medium (RPMI 1640  
containing 10% FBS, 5 X 10-5M 2ME, 100U/ml penicillin, 10ug/ml streptomycin, and  
10-5 dilution of SAC) in a total volume of 150ul. Proliferation or inhibition is quantitated  
10 by a 20h pulse (1uCi/well) with 3H-thymidine (6.7 Ci/mM) beginning 72h post factor  
addition. The positive and negative controls are IL2 and medium respectively.

In Vivo Assay- BALB/c mice are injected (i.p.) twice per day with buffer only,  
or 2 mg/Kg of a polypeptide of the invention, or truncated forms thereof. Mice receive  
15 this treatment for 4 consecutive days, at which time they are sacrificed and various tissues  
and serum collected for analyses. Comparison of H&E sections from normal spleens and  
spleens treated with polypeptides of the invention identify the results of the activity of the  
polypeptides on spleen cells, such as the diffusion of peri-arterial lymphatic sheaths,  
and/or significant increases in the nucleated cellularity of the red pulp regions, which may  
20 indicate the activation of the differentiation and proliferation of B-cell populations.  
Immunohistochemical studies using a B cell marker, anti-CD45R(B220), are used to  
determine whether any physiological changes to splenic cells, such as splenic  
disorganization, are due to increased B-cell representation within loosely defined B-cell  
25 zones that infiltrate established T-cell regions.

Flow cytometric analyses of the spleens from mice treated with polypeptide is  
used to indicate whether the polypeptide specifically increases the proportion of ThB+,  
CD45R(B220)dull B cells over that which is observed in control mice.

Likewise, a predicted consequence of increased mature B-cell representation in  
30 vivo is a relative increase in serum Ig titers. Accordingly, serum IgM and IgA levels are  
compared between buffer and polypeptide-treated mice.

One skilled in the art could easily modify the exemplified studies to test the  
activity of polynucleotides of the invention (e.g., gene therapy), agonists, and/or  
35 antagonists of polynucleotides or polypeptides of the invention.

#### **Example 42 - T Cell Proliferation Assay.**

A CD3-induced proliferation assay is performed on PBMCs and is measured by

the uptake of 3H-thymidine. The assay is performed as follows. Ninety-six well plates  
5 are coated with 100 (l/well of mAb to CD3 (HIT3a, Pharmingen) or isotype-matched  
control mAb (B33.1) overnight at 4 degrees C (1 (g/ml in .05M bicarbonate buffer, pH  
9.5), then washed three times with PBS. PBMC are isolated by F/H gradient  
centrifugation from human peripheral blood and added to quadruplicate wells (5 x  
10 104/well) of mAb coated plates in RPMI containing 10% FCS and P/S in the presence  
of varying concentrations of polypeptides of the invention (total volume 200 ul). Relevant  
protein buffer and medium alone are controls. After 48 hr. culture at 37 degrees C, plates  
are spun for 2 min. at 1000 rpm and 100 (l of supernatant is removed and stored -20  
degrees C for measurement of IL-2 (or other cytokines) if effect on proliferation is  
15 observed. Wells are supplemented with 100 ul of medium containing 0.5 uCi of 3H-  
thymidine and cultured at 37 degrees C for 18-24 hr. Wells are harvested and  
incorporation of 3H-thymidine used as a measure of proliferation. Anti-CD3 alone is the  
positive control for proliferation. IL-2 (100 U/ml) is also used as a control which  
enhances proliferation. Control antibody which does not induce proliferation of T cells  
20 is used as the negative controls for the effects of polypeptides of the invention.

One skilled in the art could easily modify the exemplified studies to test the  
activity of polynucleotides of the invention (e.g., gene therapy), agonists, and/or  
antagonists of polynucleotides or polypeptides of the invention.

25

**Example 43 - Effect Of Polypeptides Of The Invention On The Expression Of MHC  
Class II, Costimulatory And Adhesion Molecules And Cell Differentiation Of  
Monocytes And Monocyte-Derived Human Dendritic Cells.**

Dendritic cells are generated by the expansion of proliferating precursors found  
30 in the peripheral blood: adherent PBMC or elutriated monocytic fractions are cultured for  
7-10 days with GM-CSF (50 ng/ml) and IL-4 (20 ng/ml). These dendritic cells have the  
characteristic phenotype of immature cells (expression of CD1, CD80, CD86, CD40 and  
MHC class II antigens). Treatment with activating factors, such as TNF-, causes a rapid  
35 change in surface phenotype (increased expression of MHC class I and II, costimulatory  
and adhesion molecules, downregulation of FC(RII, upregulation of CD83). These  
changes correlate with increased antigen-presenting capacity and with functional  
maturation of the dendritic cells.

13 FACS analysis of surface antigens is performed as follows. Cells are treated 1-3  
5 days with increasing concentrations of polypeptides of the invention or LPS (positive  
control), washed with PBS containing 1% BSA and 0.02 mM sodium azide, and then  
incubated with 1:20 dilution of appropriate FITC- or PE-labeled monoclonal antibodies  
for 30 minutes at 4 degrees C. After an additional wash, the labeled cells are analyzed by  
flow cytometry on a FACScan (Becton Dickinson).

10 14 Effect on the production of cytokines. Cytokines generated by dendritic cells, in  
particular IL-12, are important in the initiation of T-cell dependent immune responses.  
IL-12 strongly influences the development of Th1 helper T-cell immune response, and  
induces cytotoxic T and NK cell function. An ELISA is used to measure the IL-12 release  
15 as follows. Dendritic cells (10<sup>6</sup>/ml) are treated with increasing concentrations of  
polypeptides of the invention for 24 hours. LPS (100 ng/ml) is added to the cell culture  
as positive control. Supernatants from the cell cultures are then collected and analyzed  
for IL-12 content using commercial ELISA kit(e.g., R & D Systems (Minneapolis, MN)).  
The standard protocols provided with the kits are used.

20 24 Effect on the expression of MHC Class II, costimulatory and adhesion molecules.  
Three major families of cell surface antigens can be identified on monocytes: adhesion  
molecules, molecules involved in antigen presentation, and Fc receptor. Modulation of  
the expression of MHC class II antigens and other costimulatory molecules, such as B7  
25 and ICAM-1, may result in changes in the antigen presenting capacity of monocytes and  
ability to induce T cell activation. Increase expression of Fc receptors may correlate with  
improved monocyte cytotoxic activity, cytokine release and phagocytosis.

26 FACS analysis is used to examine the surface antigens as follows. Monocytes are  
treated 1-5 days with increasing concentrations of polypeptides of the invention or LPS  
30 (positive control), washed with PBS containing 1% BSA and 0.02 mM sodium azide, and then  
incubated with 1:20 dilution of appropriate FITC- or PE-labeled monoclonal antibodies  
for 30 minutes at 4 degrees C. After an additional wash, the labeled cells are  
analyzed by flow cytometry on a FACScan (Becton Dickinson).

35 31 Monocyte activation and/or increased survival. Assays for molecules that activate  
(or alternatively, inactivate) monocytes and/or increase monocyte survival (or  
alternatively, decrease monocyte survival) are known in the art and may routinely be  
applied to determine whether a molecule of the invention functions as an inhibitor or

activator of monocytes. Polypeptides, agonists, or antagonists of the invention can be  
5 screened using the three assays described below. For each of these assays, Peripheral  
blood mononuclear cells (PBMC) are purified from single donor leukopacks (American  
Red Cross, Baltimore, MD) by centrifugation through a Histopaque gradient (Sigma).  
Monocytes are isolated from PBMC by counterflow centrifugal elutriation.

10 **Monocyte Survival Assay.** Human peripheral blood monocytes progressively lose  
viability when cultured in absence of serum or other stimuli. Their death results from  
internally regulated process (apoptosis). Addition to the culture of activating factors, such  
as TNF-alpha dramatically improves cell survival and prevents DNA fragmentation.  
Propidium iodide (PI) staining is used to measure apoptosis as follows. Monocytes are  
15 cultured for 48 hours in polypropylene tubes in serum-free medium (positive control), in  
the presence of 100 ng/ml TNF-alpha (negative control), and in the presence of varying  
concentrations of the compound to be tested. Cells are suspended at a concentration of  
2 x 10<sup>6</sup>/ml in PBS containing PI at a final concentration of 5 (g/ml, and then incubated  
at room temperature for 5 minutes before FACScan analysis. PI uptake has been  
20 demonstrated to correlate with DNA fragmentation in this experimental paradigm.

25 **Effect on cytokine release.** An important function of monocytes/macrophages is  
their regulatory activity on other cellular populations of the immune system through the  
release of cytokines after stimulation. An ELISA to measure cytokine release is  
performed as follows. Human monocytes are incubated at a density of 5x10<sup>5</sup> cells/ml  
with increasing concentrations of the a polypeptide of the invention and under the same  
conditions, but in the absence of the polypeptide. For IL-12 production, the cells are  
primed overnight with IFN (100 U/ml) in presence of a polypeptide of the invention. LPS  
(10 ng/ml) is then added. Conditioned media are collected after 24h and kept frozen until  
30 use. Measurement of TNF-alpha, IL-10, MCP-1 and IL-8 is then performed using a  
commercially available ELISA kit(e.g., R & D Systems (Minneapolis, MN)) and applying  
the standard protocols provided with the kit.

35 **Oxidative burst.** Purified monocytes are plated in 96-w plate at 2-1x10<sup>5</sup> cell/well.  
Increasing concentrations of polypeptides of the invention are added to the wells in a total  
volume of 0.2 ml culture medium (RPMI 1640 + 10% FCS, glutamine and antibiotics).  
After 3 days incubation, the plates are centrifuged and the medium is removed from the  
wells. To the macrophage monolayers, 0.2 ml per well of phenol red solution (140 mM

5 NaCl, 10 mM potassium phosphate buffer pH 7.0, 5.5 mM dextrose, 0.56 mM phenol red and 19 U/ml of HRPO) is added, together with the stimulant (200 nM PMA). The plates are incubated at 37°C for 2 hours and the reaction is stopped by adding 20 µl 1N NaOH per well. The absorbance is read at 610 nm. To calculate the amount of H<sub>2</sub>O<sub>2</sub> produced by the macrophages, a standard curve of a H<sub>2</sub>O<sub>2</sub> solution of known molarity is performed for each experiment.

10 One skilled in the art could easily modify the exemplified studies to test the activity of polynucleotides of the invention (e.g., gene therapy), agonists, and/or antagonists of polynucleotides or polypeptides of the invention.

15 **Example 44 - Biological Effects of HGPRBMY2 Polypeptides of the Invention.**

Astrocyte and Neuronal Assays.

20 Recombinant polypeptides of the invention, expressed in *Escherichia coli* and purified as described above, can be tested for activity in promoting the survival, neurite outgrowth, or phenotypic differentiation of cortical neuronal cells and for inducing the proliferation of glial fibrillary acidic protein immunopositive cells, astrocytes. The selection of cortical cells for the bioassay is based on the prevalent expression of FGF-1 and FGF-2 in cortical structures and on the previously reported enhancement of cortical neuronal survival resulting from FGF-2 treatment. A thymidine incorporation assay, for 25 example, can be used to elucidate a polypeptide of the invention's activity on these cells.

25 Moreover, previous reports describing the biological effects of FGF-2 (basic FGF) on cortical or hippocampal neurons *in vitro* have demonstrated increases in both neuron survival and neurite outgrowth (Walicke et al., "Fibroblast growth factor promotes survival of dissociated hippocampal neurons and enhances neurite extension." 30 Proc. Natl. Acad. Sci. USA 83:3012-3016. (1986), assay herein incorporated by reference in its entirety). However, reports from experiments done on PC-12 cells suggest that these two responses are not necessarily synonymous and may depend on not only which FGF is being tested but also on which receptor(s) are expressed on the target cells. Using the 35 primary cortical neuronal culture paradigm, the ability of a polypeptide of the invention to induce neurite outgrowth can be compared to the response achieved with FGF-2 using, for example, a thymidine incorporation assay.

Fibroblast and endothelial cell assays.

5 Human lung fibroblasts are obtained from Clonetics (San Diego, CA) and maintained in growth media from Clonetics. Dermal microvascular endothelial cells are obtained from Cell Applications (San Diego, CA). For proliferation assays, the human lung fibroblasts and dermal microvascular endothelial cells can be cultured at 5,000 cells/well in a 96-well plate for one day in growth medium. The cells are then incubated  
10 for one day in 0.1% BSA basal medium. After replacing the medium with fresh 0.1% BSA medium, the cells are incubated with the test proteins for 3 days. Alamar Blue (Alamar Biosciences, Sacramento, CA) is added to each well to a final concentration of 10%. The cells are incubated for 4 hr. Cell viability is measured by reading in a  
15 CytoFluor fluorescence reader. For the PGE2 assays, the human lung fibroblasts are cultured at 5,000 cells/well in a 96-well plate for one day. After a medium change to 0.1% BSA basal medium, the cells are incubated with FGF-2 or polypeptides of the invention with or without IL-1( for 24 hours. The supernatants are collected and assayed for PGE2 by EIA kit (Cayman, Ann Arbor, MI). For the IL-6 assays, the human lung  
20 fibroblasts are cultured at 5,000 cells/well in a 96-well plate for one day. After a medium change to 0.1% BSA basal medium, the cells are incubated with FGF-2 or with or without polypeptides of the invention IL-1( for 24 hours. The supernatants are collected and assayed for IL-6 by ELISA kit (Endogen, Cambridge, MA).

25 Human lung fibroblasts are cultured with FGF-2 or polypeptides of the invention for 3 days in basal medium before the addition of Alamar Blue to assess effects on growth of the fibroblasts. FGF-2 should show a stimulation at 10 - 2500 ng/ml which can be used to compare stimulation with polypeptides of the invention.

30 Parkinson Models.

The loss of motor function in Parkinson's disease is attributed to a deficiency of striatal dopamine resulting from the degeneration of the nigrostriatal dopaminergic projection neurons. An animal model for Parkinson's that has been extensively characterized involves the systemic administration of 1-methyl-4 phenyl 1,2,3,6-35 tetrahydropyridine (MPTP). In the CNS, MPTP is taken-up by astrocytes and catabolized by monoamine oxidase B to 1-methyl-4-phenyl pyridine (MPP+) and released. Subsequently, MPP+ is actively accumulated in dopaminergic neurons by the high-

affinity reuptake transporter for dopamine. MPP<sup>+</sup> is then concentrated in mitochondria 5 by the electrochemical gradient and selectively inhibits nicotidamide adenine disphosphate: ubiquinone oxidoreductionase (complex I), thereby interfering with electron transport and eventually generating oxygen radicals.

It has been demonstrated in tissue culture paradigms that FGF-2 (basic FGF) has 10 trophic activity towards nigral dopaminergic neurons (Ferrari et al., Dev. Biol. 1989). Recently, Dr. Unsicker's group has demonstrated that administering FGF-2 in gel foam implants in the striatum results in the near complete protection of nigral dopaminergic neurons from the toxicity associated with MPTP exposure (Otto and Unsicker, J. Neuroscience, 1990).

15 Based on the data with FGF-2, polypeptides of the invention can be evaluated to determine whether it has an action similar to that of FGF-2 in enhancing dopaminergic neuronal survival in vitro and it can also be tested in vivo for protection of dopaminergic neurons in the striatum from the damage associated with MPTP treatment. The potential effect of a polypeptide of the invention is first examined in vitro in a dopaminergic 20 neuronal cell culture paradigm. The cultures are prepared by dissecting the midbrain floor plate from gestation day 14 Wistar rat embryos. The tissue is dissociated with trypsin and seeded at a density of 200,000 cells/cm<sup>2</sup> on polyorthinine-laminin coated glass coverslips. The cells are maintained in Dulbecco's Modified Eagle's medium and F12 25 medium containing hormonal supplements (N1). The cultures are fixed with paraformaldehyde after 8 days in vitro and are processed for tyrosine hydroxylase, a specific marker for dopaminergic neurons, immunohistochemical staining. Dissociated cell cultures are prepared from embryonic rats. The culture medium is changed every third day and the factors are also added at that time.

30 Since the dopaminergic neurons are isolated from animals at gestation day 14, a developmental time which is past the stage when the dopaminergic precursor cells are proliferating, an increase in the number of tyrosine hydroxylase immunopositive neurons would represent an increase in the number of dopaminergic neurons surviving in vitro. 35 Therefore, if a polypeptide of the invention acts to prolong the survival of dopaminergic neurons, it would suggest that the polypeptide may be involved in Parkinson's Disease.

One skilled in the art could easily modify the exemplified studies to test the activity of polynucleotides of the invention (e.g., gene therapy), agonists, and/or

antagonists of polynucleotides or polypeptides of the invention.

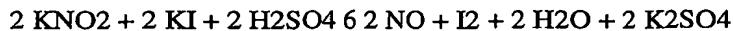
5

**Example 45 - Stimulation Of Nitric Oxide Production By Endothelial Cells.**

Nitric oxide released by the vascular endothelium is believed to be a mediator of vascular endothelium relaxation. Thus, activity of a polypeptide of the invention can be assayed by determining nitric oxide production by endothelial cells in response to the 10 polypeptide.

Nitric oxide is measured in 96-well plates of confluent microvascular endothelial cells after 24 hours starvation and a subsequent 4 hr exposure to various levels of a positive control (such as VEGF-1) and the polypeptide of the invention. Nitric oxide in 15 the medium is determined by use of the Griess reagent to measure total nitrite after reduction of nitric oxide-derived nitrate by nitrate reductase. The effect of the polypeptide of the invention on nitric oxide release is examined on HUVEC.

Briefly, NO release from cultured HUVEC monolayer is measured with a NO-specific polarographic electrode connected to a NO meter (Iso-NO, World Precision 20 Instruments Inc.) (1049). Calibration of the NO elements is performed according to the following equation:



The standard calibration curve is obtained by adding graded concentrations of 25  $\text{KNO}_2$  (0, 5, 10, 25, 50, 100, 250, and 500 nmol/L) into the calibration solution containing KI and  $\text{H}_2\text{SO}_4$ . The specificity of the Iso-NO electrode to NO is previously determined by measurement of NO from authentic NO gas (1050). The culture medium is removed and HUVECs are washed twice with Dulbecco's phosphate buffered saline. The cells are then bathed in 5 ml of filtered Krebs-Henseleit solution in 6-well plates, and 30 the cell plates are kept on a slide warmer (Lab Line Instruments Inc.) To maintain the temperature at 37°C. The NO sensor probe is inserted vertically into the wells, keeping the tip of the electrode 2 mm under the surface of the solution, before addition of the different conditions. S-nitroso acetyl penicillamin (SNAP) is used as a positive control. The amount of released NO is expressed as picomoles per  $1 \times 10^6$  endothelial cells. All 35 values reported are means of four to six measurements in each group (number of cell culture wells). See, Leak et al. Biochem. and Biophys. Res. Comm. 217:96-105 (1995).

One skilled in the art could easily modify the exemplified studies to test the

activity of polynucleotides of the invention (e.g., gene therapy), agonists, and/or  
5 antagonists of polynucleotides or polypeptides of the invention.

**Example 46 - Effect Of Polypeptides Of The Invention On Vasodilation.**

Since dilation of vascular endothelium is important in reducing blood pressure,  
10 the ability of polypeptides of the invention to affect the blood pressure in spontaneously  
hypertensive rats (SHR) is examined. Increasing doses (0, 10, 30, 100, 300, and 900  
mg/kg) of the polypeptides of the invention are administered to 13-14 week old  
spontaneously hypertensive rats (SHR). Data are expressed as the mean +/- SEM.  
Statistical analysis are performed with a paired t-test and statistical significance is defined  
15 as p<0.05 vs. the response to buffer alone.

One skilled in the art could easily modify the exemplified studies to test the  
activity of polynucleotides of the invention (e.g., gene therapy), agonists, and/or  
antagonists of polynucleotides or polypeptides of the invention.

20 **Example 47 - Peripheral Arterial Disease Model.**

Angiogenic therapy using a polypeptide of the invention is a novel therapeutic  
strategy to obtain restoration of blood flow around the ischemia in case of peripheral  
arterial diseases. The experimental protocol includes:

25 a) One side of the femoral artery is ligated to create ischemic muscle of  
the hindlimb, the other side of hindlimb serves as a control.

b) a polypeptide of the invention, in a dosage range of 20 mg - 500 mg, is  
delivered intravenously and/or intramuscularly 3 times (perhaps more) per week for 2-3  
weeks.

30 c) The ischemic muscle tissue is collected after ligation of the femoral  
artery at 1, 2, and 3 weeks for the analysis of expression of a polypeptide of the invention  
and histology. Biopsy is also performed on the other side of normal muscle of the  
contralateral hindlimb.

35 One skilled in the art could easily modify the exemplified studies to test the  
activity of polynucleotides of the invention (e.g., gene therapy), agonists, and/or  
antagonists of polynucleotides or polypeptides of the invention.

**Example 48 - Ischemic Myocardial Disease Model.**

5        A polypeptide of the invention is evaluated as a potent mitogen capable of stimulating the development of collateral vessels, and restructuring new vessels after coronary artery occlusion. Alteration of expression of the polypeptide is investigated in situ. The experimental protocol includes:

10      a) The heart is exposed through a left-side thoracotomy in the rat. Immediately, the left coronary artery is occluded with a thin suture (6-0) and the thorax is closed.

      b) a polypeptide of the invention, in a dosage range of 20 mg - 500 mg, is delivered intravenously and/or intramuscularly 3 times (perhaps more) per week for 2-4 weeks.

15      c) Thirty days after the surgery, the heart is removed and cross-sectioned for morphometric and in situ analyzes.

      One skilled in the art could easily modify the exemplified studies to test the activity of polynucleotides of the invention (e.g., gene therapy), agonists, and/or antagonists of polynucleotides or polypeptides of the invention.

20

**EQUIVALENTS AND REFERENCES**

      Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments of the invention described herein. Indeed, various modifications of the invention in addition to those described herein will become apparent to those skilled in the art from the foregoing description and accompanying figures. Such equivalents are intended to be within the scope of the following claims.

      All publications, patents and patent applications mentioned in this specification 30 are herein incorporated by reference into the specification to the same extent as if each individual publication, patent or patent application was specifically and individually indicated to be incorporated herein by reference.

35

What is claimed is:

- 5 1. An isolated nucleic acid molecule consisting of a polynucleotide having a nucleotide sequence selected from the group consisting of:
  - (a) a polynucleotide encoding a polypeptide of SEQ ID NO:2;
  - (b) an isolated polynucleotide consisting of nucleotides 250 to 1323 of SEQ ID NO:1, wherein said nucleotides encode a polypeptide corresponding to amino acids 2 to 359 of SEQ ID NO:2 minus the start codon;
  - (c) an isolated polynucleotide consisting of nucleotides 247 to 1323 of SEQ ID NO:1, wherein said nucleotides encode a polypeptide corresponding to amino acids 1 to 329 of SEQ ID NO:2 including the start codon;
  - 15 (d) a polynucleotide encoding the HGPRBMY1 polypeptide encoded by the cDNA clone contained in ATCC Deposit No. XXXXX;
  - (e) a polynucleotide which represents the complimentary sequence (antisense) of SEQ ID NO:1;
    - (a) a polynucleotide encoding a polypeptide of SEQ ID NO:14;
    - (b) an isolated polynucleotide consisting of nucleotides 362 to 1651 of SEQ ID NO:13, wherein said nucleotides encode a polypeptide corresponding to amino acids 2 to 431 of SEQ ID NO:14 minus the start codon;
    - (c) an isolated polynucleotide consisting of nucleotides 359 to 1651 of SEQ ID NO:13, wherein said nucleotides encode a polypeptide corresponding to amino acids 1 to 431 of SEQ ID NO:13 including the start codon;
    - 25 (d) a polynucleotide encoding the HGPRBMY2 polypeptide encoded by the cDNA clone contained in ATCC Deposit No. XXXXX; and
    - (e) a polynucleotide which represents the complimentary sequence (antisense) of SEQ ID NO:13.
- 30 2. The isolated nucleic acid molecule of claim 1, wherein the polynucleotide comprises a nucleotide sequence encoding a human G-protein coupled receptor protein.
- 35 3. A recombinant vector comprising the isolated nucleic acid molecule of claim 2.
4. A recombinant host cell comprising the recombinant vector of claim 3.

5. An isolated polypeptide consisting of an amino acid sequence selected from the group consisting of:
  - (a) a full length protein of SEQ ID NO:2;
  - (b) a polypeptide corresponding to amino acids 2 to 359 of SEQ ID NO:2, wherein said amino acids 2 to 359 comprise a polypeptide of SEQ ID NO:2 minus the start methionine;
  - 10 (c) a polypeptide corresponding to amino acids 1 to 359 of SEQ ID NO:2;
  - (d) a polypeptide encoded by the cDNA contained in ATCC Deposit No. XXXXXX;
  - (e) a full length protein of SEQ ID NO:14;
  - 15 (f) a polypeptide corresponding to amino acids 2 to 431 of SEQ ID NO:14, wherein said amino acids 2 to 431 comprise a polypeptide of SEQ ID NO:14 minus the start methionine;
  - (g) a polypeptide corresponding to amino acids 1 to 431 of SEQ ID NO:14; and
  - 20 (h) a polypeptide encoded by the cDNA contained in ATCC Deposit No. XXXXXX.
6. A cell comprising the polypeptide of claim 4 and a member selected from the group consisting of NFAT/CRE, and NFAT G alpha 15.
- 25 7. A method of screening for candidate compounds capable of modulating activity of a G-protein coupled receptor-encoding polypeptide, comprising:
  - (a) contacting a test compound with the cell according to claim 6; and
  - (b) selecting as candidate modulating compounds those test compounds that
- 30 8. A polypeptide that binds to the polypeptide of claim 1 selected from the group consisting of SEQ ID NO:32, 33, 34, and 35.
9. The polypeptide of claim 8 wherein said polypeptide modulates the G-protein coupled receptor of claim 5.

10. A method of screening for candidate compounds capable of modulating  
5 activity of a G-protein coupled receptor-encoding polypeptide,  
comprising:

(a). contacting the modulating polypeptide of claim 9 with the cell  
according to claim 4;

10 (b). contacting a test compound with said cell; and

(c). selecting as candidate modulating compounds those test  
compounds that modulate activity of the G-protein coupled receptor polypeptide.

11. A method of modulating the G-protein coupled receptor activity of the  
polypeptide of claim 5 comprising an effective amount of the polypeptide  
15 of claim 9.

12. The method for the treatment of immune-related disorders comprising a  
member of the group consisting of:

20 (a) administering to the subject a therapeutically effective  
amount of a HGPRBMY1 polypeptide;

(b) modulating the activity of a HGPRBMY1 polypeptide;

25 (c) administering to the subject a therapeutically effective  
amount of a HGPRBMY1 polypeptide wherein the  
HGPRBMY1 polypeptide is contained in a  
pharmaceutical composition;

(d) modulating the activity of a HGPRBMY1 polypeptide  
wherein the HGPRBMY1 polypeptide is HGPRBMY1 or  
a functionally equivalent derivative thereof;

30 (e) wherein the HGPRBMY1 polypeptide is HGPRBMY1 or  
a functionally equivalent derivative thereof wherein the  
HGPRBMY1 polypeptide is HGPRBMY1 or a  
functionally equivalent derivative thereof wherein the  
method comprises administering an effective amount of a  
compound that agonizes or antagonizes the activity of the  
HGPRBMY1 polypeptide;

35 (f) administering an effective amount of a compound that  
decreases expression of a HGPRBMY1 gene;

5 (g) administering an effective amount of a compound that decreases expression of a HGPRBMY1 gene in which the compound is an oligonucleotide encoding an antisense or ribozyme molecule that targets HGPRBMY1 transcripts and inhibits translation; and

10 (h) administering an effective amount of a compound that increases expression of a HGPRBMY1 gene.

13. The method for the treatment of heart-related disorders comprising a member of the group consisting of:

15 (i) administering to the subject a therapeutically effective amount of a HGPRBMY2 polypeptide;

(j) modulating the activity of a HGPRBMY2 polypeptide;

20 (k) administering to the subject a therapeutically effective amount of a HGPRBMY2 polypeptide wherein the HGPRBMY2 polypeptide is contained in a pharmaceutical composition;

(l) modulating the activity of a HGPRBMY2 polypeptide wherein the HGPRBMY2 polypeptide is HGPRBMY2 or a functionally equivalent derivative thereof;

25 (m) wherein the HGPRBMY2 polypeptide is HGPRBMY2 or a functionally equivalent derivative thereof wherein the HGPRBMY2 polypeptide is HGPRBMY2 or a functionally equivalent derivative thereof wherein the method comprises administering an effective amount of a compound that agonizes or antagonizes the activity of the HGPRBMY2 polypeptide;

30 (n) administering an effective amount of a compound that decreases expression of a HGPRBMY2 gene;

35 (o) administering an effective amount of a compound that decreases expression of a HGPRBMY2 gene in which the compound is an oligonucleotide encoding an antisense or



5           18. The method of preventing, treating, or ameliorating a medical condition  
          of claim 14, wherein the medical condition is a metabolic disorder,  
          obesity, and pain.

10           19. The method of preventing, treating, or ameliorating a medical condition  
          of claim 14, wherein the condition is a condition related to aberrant cell  
          cycle regulation, aberrant p27 regulation, aberrant apoptosis regulation,  
          aberrant I<sub>K</sub>B regulation, aberrant NF<sub>k</sub>B regulation, aberrant DNA repair,  
          and aberrant cellular phosphorylation.

15           20. The isolated polypeptide of claim 5, wherein the full length protein  
          comprises sequential amino acid deletions from either the C-terminus or  
          the N-terminus.

20

25

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GGCCGCCTTT GCAAGGTTGC TGGACAGATG GAACTGGAAG GGCAGCCGTC  
TGCCGCCAAC GAACACCTTC TCAAGCACTT TGAGTGACCA CGGCTTGCAA  
GCTGGTGGCT GGCCCCCGA GTCCCGGGCT CTGAGGCACG GCCGTCGACT  
TAAGCGTTGC ATCCTGTTAC CTGGAGACCC TCTGAGCTCT CACCTGCTAC  
TTCTGCCGCT GCTTCTGCAC AGAGCCCGGG CGAGGACCCC TCCAGG

ATGCAGGTCC CGAACAGCAC CGGCCCAGAC AACCGCACGC TGCAGATGCT  
GCGGAACCCG GCGATCGCGG TGGCCCTGCC CGTGGTGTAC TCGCTGGTGG  
CGGGGTCAG CATCCCCGGC AACCTCTTCT CTCTGTGGGT GCTGTGCCGG  
CGCATGGGGC CCAGATCCCC GTCGGTATCA TTCATGATCA ACCTGAGCGT  
CACGGACCTG ATGCTGGCCA GCGTGTGCC TTTCCAAATC TACTACCATT  
GCAACCGCCA CCACTGGGTA TTCGGGGTGC TGCTTTGCAA CGTGGTGACC  
GTGGCCTTTT ACGCAAACAT GTATTCCAGC ATCCTCACCA TGACCTGTAT  
CAGCGTGGAG CGCTTCCTGG GGGTCCTGTA CCCGCTCAGC TCCAAGCGCT  
GGCGCCGCCG TCGTTACGCG GTGGCCGCGT GTGCAGGGAC CTGGCTGCTG  
CTCCTGACCG CCCCTGCCCC GCTGGCCGCG ACCGATCTCA CCTACCCGGT  
GCACGCCCTG GGCATCATCA CCTGCTTCGA CGTCCCTCAAG TGGACGATGC  
TCCCCAGCGT GGCCATGTGG GCCGTGTTCC TCTTCACCAT CTTCATCCTG  
CTGTTCTCA TCCCCTTCGT GATCACCGTG GCTGTGTACA CGGCCACCAT  
CCTCAAGCTG TTGCGCACGG AGGAGGCGCA CGGGCGGGAG CAGCGGAGGC  
GCGGGTGGG CCTGGCCGCG GTGGTCTTGC TGGCCTTTGT CACCTGCTTC  
GCCCCCAACA ACTTCGTGCT CCTGGCGCAC ATCGTGAGCC GCCTGTTCTA  
CGGCAAGAGC TACTACCACG TGTACAAGCT CACCGCTGTGT CTCAGCTGCC  
TCAACAACTG TCTGGACCCG TTTGTTTATT ACTTTGCGTC CGGGGAATTTC  
CAGCTGCGCC TGCAGGAAATA TTTGGGCTGC CGCCGGGTGC CCAGAGACAC  
CCTGGACACG CGCCGCGAGA GCCTCTTCTC CGCCAGGACC ACGTCCGTGC  
GCTCCGAGGC CGGTGCGCAC CCTGAAGGGGA TGGAGGGAGC CACCAGGCC  
GGCCTCCAGA GGCAGGAGAG TGTGTTC

TCCCTGCTGA CATCGTCCCT TAGTTGTGGT TCTGGCCTTC TCCATTCTCC  
TCCAGGGGTT CTGGTCTCCG TAGCCCGGGT CACGCCGAAA TTTCTGTTA  
TTTCACTCAG GGGCACTGTG GTTGCTGTGG TTGGAATTCT TCTTCAGAG  
GAGGCCCTGG GGCTCCTGCA AGTCAGCTAC TCTCCGTGCC CACTTCCCT  
CACACACACA CCCCCCTCGT GCCGAATTCT T

**FIG. 1**

MQVPNSTGPDNATLQMLRNPAIAVALPVVYSLVAAVSIPGNLFSLWVLCRRMGRSPSPVI  
FMINLSVTDLMLASVLPFQIYYHCNRHHWVFGVLLCNVVTVAFYANMYSSILTMTCISVE  
RFLGVLYPLSSKRWRRRYAVAACAGTWLLLLTALSPLARTDLTYPVHALGIITCFDVLK  
WTMLPSVAMWAVFLFTIFILLFLIPFVITVACYTATILKLLRTEEAHGREQRRRAVGLAA  
VVLLAFVTCFAPNNFVLLAHIVSRLFYGKSYYHVKLTLCCLSCLNNCLDPFVYYFASREF  
QLRLREYLGCRRVPRDTLDTRRESLFSAARTTSVRSEAGAHPEGMEGATRPGLQRQESVF

**FIG. 2**

1 MQVPNSTGPD NATLQMLRNP AIAVALPVVV SLVAAVSIPG NLFSLWVLCR  
51 RMGPRSPSPVI FMINLSVTDL MLASVLPFQI YYHCNRHHWV FGVLLCNVVT  
101 VAFYANMYSS ILTMTCISVE RFLGVLYPLS SKRWRRRYA VAACAGTWLL  
151 LLTALSPLAR TDLTYPVHAL GIITCFDVLK WTMLPSVAMW AVFLFTIFIL  
201 LFLIPFVITV ACYTATILKL LRTEEAHGRE QRRAVGLAA VVLLAFVTCF  
251 APNNFVLLAH IVSRLFYGKS YYHVYKLTLC LSCLNNCLDP FVYYFASREF  
301 QLRLREYLGC RRVPRDTLDT RRESLFSART TSVRSEAGAH PEGMEGATRP  
351 GLQRQESVF

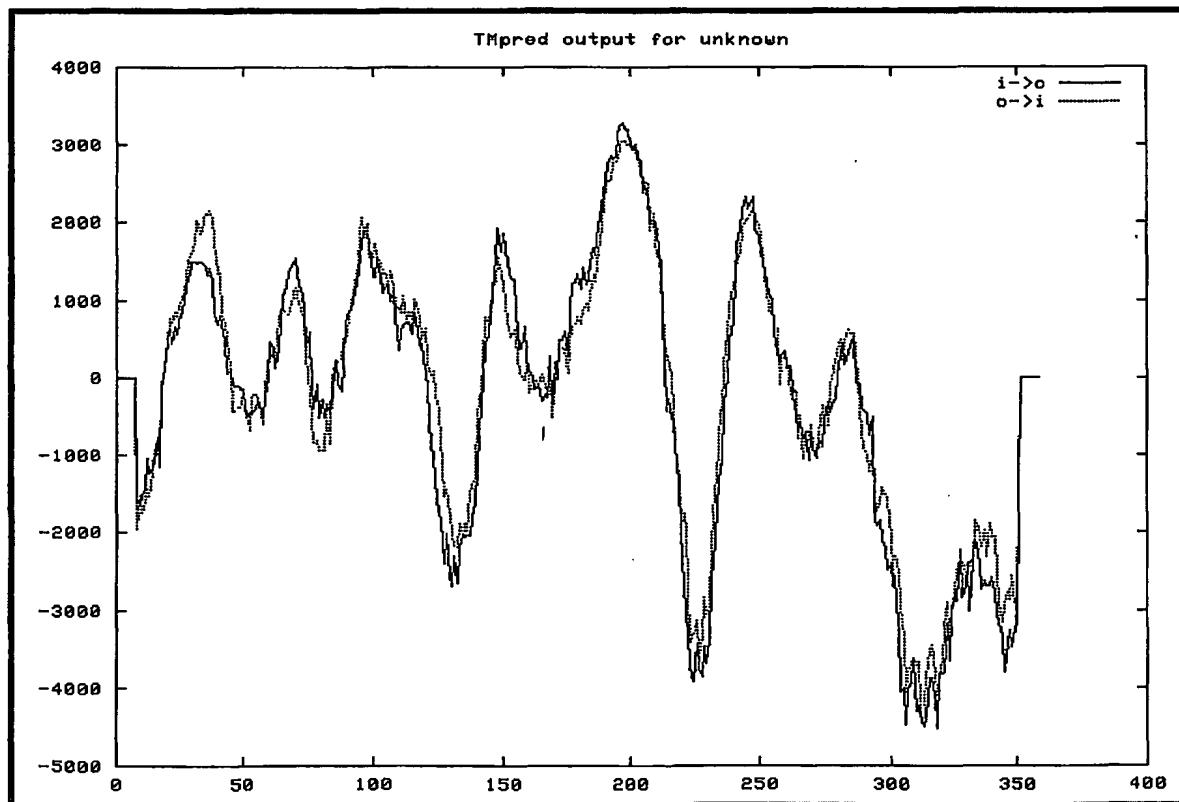


FIG. 3

par2_human	-----MRSPSAWLLGAA. ILLAASLSC. SGTIQGTONRSSKGRSLIGKVD. .GT.....
par3_human	-----MKALIIFAAAGLILLLLPTFCOSGMENDTNNLAKPTLPIKTFR. .GAPPNSF
thrombin_Xeno	MMELRVLLLLLLTLIGAMGSLC. .LANSDTQAKGAHS. .NNMTH. .KTFRIFDDSESEF
thrombin_human	-----MGPRRLIIVAACPSLCGPLSARTRARRPESKATNATEDPRSF. LLRNPNDKY
HGPRBMY1	-----
par4_human	-----MWGRLLIWPLVLGFSLSGGTQT
p2y9_human	-----
par2_human	.....SHVT.G.KGVTVETVFSVDEFSAS. ....
par3_human	EEFPFSALE.GWTGATITVKKKCPPEESASHLH.V. ....K. NATMG. .YLISSLSTK
thrombin_Xeno	EEIPWDELDESGEQSGDQAPVRSARKPIRRN. ....ITKE. .AE.Q. .YLISSLWLT
thrombin_human	EPF.WEDEEKNESGLDEYRLA.SINKSSPLQKD. LPAFISED. .AS.G. .YLISSLWLT
HGPRBMY1	-----M9. VPNSTGPD. .NATLQ. .MIRNPAAIV
par4_human	PSVYDESGSTGGDDSTPSIIPAPRGYPGQVC. ANDSDTLELPDSSRA. .LLLGWVPTR
p2y9_human	-----MGDERRFIDFQFODSNSSLRPRLGNATANNTCIVDDSFKYN
par2_human	FIPIVYTTIVFVVLPSNGQALWVHLFRKKKHPAVTYMANLADLLSVIWFPLKIAYH
par3_human	LPATIYLLVVFVVGVPANAVLWMLLFFRTRSICTTVEY. TNLAIAADELFCVTLPPFKIAYH
thrombin_Xeno	FVPSLYTVVFIVGLPLNLIAKELFLERMKVRKPAVVYMLNLADVEFVSVLPFPKIAYH
thrombin_human	FVPSVYIGVVFVVSPLPLNIMAVIVWEILAKMKVKKPAVVYMIHLATADVLFVSVLPFPKISYYF
HGPRBMY1	AVPVVYSTVAAVSIPGNLFLSLWVLCRMRGPRSPSIVFMINLSVTDLMLASVLPFQIYYHC
par4_human	LPVALLYGLIVLVVGLPANGHALWVLATQA. PRLPSTMLLMNLTADLLLALALPERIAYH
p2y9_human	LNGAVYSVVFIIGLITNSMSLVECFRRMKPSETAIFITNLAVSDLLFVCTLPPFKIFYNF
par2_human	HGNNWVYGEALCNVHLIGFFYCNMYCISILFWTCLSVORYWVIVNPMGH. SRRKANIAALGIS
par3_human	NCNNWVFCGVLCRATTIVFYCNMYCISILLIACISHEFRLAIVHPFTYRGLPKHTYALAVTC
thrombin_Xeno	SGNDWLFGPGMCRTVTAIFYCNMYCISILLIASISVDRFLAVVYPMHSLSWRTMSRAYMAC
thrombin_human	SGSDWQFGSELCREVTAAFYCNMYCISILLMTVISIDRFLAVVYPMOSLSWRTLGRASFTC
HGPRBMY1	NRHHWVFGVLLCNVVTVAFYANMYSSILIMTCISVERFLGVLYPLSSKRWERRRYAAAC
par4_human	RCQRWPFGHAACRILATAALYGHMYGSLLIAAVSFLDRYLALVHPURALARGRRLALGLC
p2y9_human	N.RHWPFGDTLCKISGTAAFLTMIVGSMFLUTCISVDRFLATIVYPRSRPTIIRRNSAIVC
par2_human	LAIWLLIILVTEPLIVVKOTIFIPALINITTCHDV. .LPEQLLVDMDENY. FLSLA. .EGVF
par3_human	GLNWATMFLYMLPFFFLKQEEYIVQPDITTCVDHNTCESSSPFQLY. Y. FISLA. FFGF
thrombin_Xeno	SFIWLIESTASTIPPLATEQTKSPREDITTCVDVLDLKD. .FYIIVYBSSPC. .LFF
thrombin_human	LAIWALATAGVIVPLVILKEOTIQVPGINITTCHDVNLNETLLEG. .YYAVYESAENS. AVFF
HGPRBMY1	AGTWLLIILTALSPLARTDLITYVHALGIIITCFDVLKWTMLPSVAMWAVEFLTIE. .SFLF
par4_human	MAIWLMAAAALAPLTLQRQTFRIARSDRVLCHDALP. .LDAQASHMQPAFTCLA. .LGC
p2y9_human	AGSMWILVISSGTISASLFST. .NWNAT. .TTCFEGFS. .KRVWKTYLSSKIIIFIEWGF
par2_human	LFPAPFLTASAYV. LMIRMRSSAMDENSEKKRKRAIKLIVTVLAMYLIICFTPSNELLVVA
par3_human	LPFVLLIBYCYA. AIIRTL. .NAYDH. .RWLWYVKASLILVIFTICFAPSNLILLI
thrombin_Xeno	FVPFPLTTTCYIG. IIIRSLSSSSIENSCKNT. .RALFLAVVVLCVFIIICRGPTNVLFLTH
thrombin_human	FVPLTITSTVCYVS. IIRCOLSSSSAVERSKE. .RALFLSAAVFCIIFIICFGPTNVLLLAH
HGPRBMY1	LIPEVITVACYATIIEKLLRTEEAHGREORK. .RAVGLAAVLLAFLVTCAPNNEVLLAH
par4_human	FEPILLAMLLCY. GATEHTLAASG. .RYGHALRLTAVVLLASAVAFVFPNSNLLLLH
p2y9_human	IIPLTITVNSC. SSVVILRLRKPAATLSQIGTNKKVLMITVHMAVFWVCFVEYNSVDFLY

FIG. 4

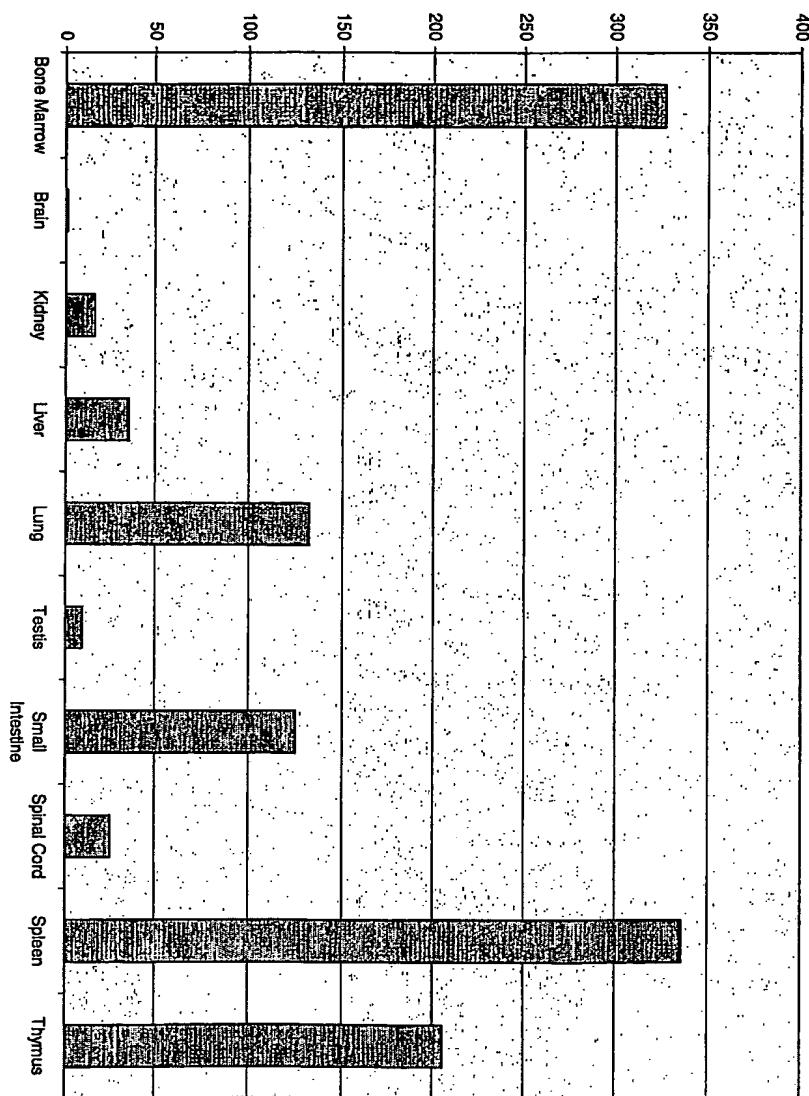
par2\_human  
par3\_human  
thrombin\_Xeno  
thrombin\_human  
HGPRBMY1  
par4\_human  
p2y9\_human

YFLD. KSQGQ. . . . SHVYALYIVALCLSTLNSQIDPFLVYYFVSHDFRDHAKNALLRSVR  
HANY. YYNNT. . . . DGLYFYIYLIALCLGSLNSQIDPFLYFLMSKT. RNHS. TAYLTK~~~  
Y. EQEAN. . . . EFLYFAYILSACVGSYSCGLDPLIYYFASSECQRYFYSLLCCRKVS  
YSFISHTSTT. . . . EAAFYAYLLCVCVSSSISSCIDPFLIYYFASSECQRYFYSILCCGESS  
.. IVSRLFYG. . . . KSYYHVVKLTLCSCLNNCIDPFLVYFASREFOLREREYLGCCR. .  
YSDPSPSAWG. . . . N. EYGAIVPSLALSILNSQIDPFIYYFVSAEFRDKVRAGLFQFSPG  
ALVRSQAITNCFLERFAKIVYPTILCLATLNCGFDPFIYYFLESFQKSFYINAHIRMES

par2\_human  
par3\_human  
thrombin\_Xeno  
thrombin\_human  
HGPRBMY1  
par4\_human  
p2y9\_human

TVKQMQVETLTSKKHSRQSSSYSSSSTTVKTSY~~~  
~~~  
EPGS. . . SGQLMSTAMNDNCSTNAKSSSIYKKLTA~~~  
DPSSYNSSGQLM. . ASKMDTCSSNLNNSIYKKLTT~~~  
. PRDTLDIIRRESLFSAR. . ITTSVRSEAGAHPEGMEGATRPGLQRQESVF  
DTVASKASAEGGSRGMGTHSSLLO~~~  
LFKTETPLTTKPSLPAIQEEVSDOTTNNGGELMESTF~~~

**FIG. 4 Continued**

**FIG. 5**

CCACCGCGTCCGGGCGCCAGGACCCCTAGCGTGGCGCTCCAGCACCCAGACCGTGGCGCG  
CCTCGCCCTAGGAAAGAGCAAGGGAAGAACATTATTGAACCGCGAACATTGGTCA  
CTGAGATCGAGTCCTCCAGTGCTTGGCTCCCGCTCTTATCTGGGTTGATCCCTG  
AGCTGCTCTCCTTCCGAACCTCCGGGGTGAGCCTAGAGCCCTCCCGCGGGCTGAC  
TCCAGAGTAGAGGAAGGGAGGCAGGCCAGTGCGGGCGGTGGTCCCCCGAAGCCCTCGCTGCCCCGC  
AGATGCGGATGGCCAGCCAGTAGCGGGCGGTGGCCCCCGCGTCCCCGGAGCGCACAGCA

ATGCAGGCGCTTAACATTACCCCGGAGCAGTTCTCTCGGCTGCTGCGGGACCACAACCTG  
ACCGGGAGCAGTTATCGCTCTGTACCGGCTGCGACCCTCGTACACCCCCAGAGCTG  
CCGGGACGCGCCAAGCTGGCCCTCGTGCACCCGGCTGCTCATCTCGCCCTGGCGCTC  
TTTGGCAATGCTCTGGTGTCTACGTGGTGACCCGAGCAAGGCCATGCGCACCGTACC  
AACATCTTATCTGCTCTGGCGTCAGTGACCTGCTCATCACCTCTTCTGCATTCC  
GTCACCATGCTCCAGAACATTCCGACAACCTGGCTGGGGGTGCTTCATTGCAAGATG  
GTGCCATTGTCCAGTCTACCGCTGTGTGACAGAAATCCTCACTATGACCTGCATTGCT  
GTGGAAAGGCACCAGGGACTTGTGCATCCTTAAAATGAAGTGGCAATACACCAACCGA  
AGGGCTTTACAATGCTAGGTGTGGCTGGCTGGCAGTCATCGTAGGATCACCCATG  
TGGCACGTGCAACAACTTGAGATCAAATGACTTCCTATATGAAAAGGAACACATCTGC  
TGCTTAGAAGAGTGGACAGCCCTGTGCACCAAGAGATCTACACCACCTTCATCCTTGTG  
ATCCTCTCCTCCCTGCCTCTTATGGTGTGCTATTCTGTACAGTAAAATTGGTTATGAA  
CTTGGATAAAAGAAAAGAGTTGGGGATGGTTCACTGCTGAACATTATGAAAGAA  
ATGTCCAAAATAGCCAGGAAGAAGAACGAGCTGTCATTATGATGTCAGTGACAGTGGTGGCT  
CTCTTGCTGTGCTGGCACCATTCATGTTGTCATATGATGATTGAATACAGTAAT  
TTTGGAAAAGGAATATGATGATGTACAATCAAGATGATTTTGCTATCGTCAAATTATT  
GGATTTCCAACCTCCATCTGTAATCCATTGCTATGCATTATGAAAGAAACTTCAA  
AAAATGTTGTGCTGCAGTTGTATTGCATAGTAAATAAAACCTCTCCAGCACAA  
AGGCATGGAATTCAAGAATTACAATGATGCGGAAGAAAGCAAAAGTTTCCCTCAGAGAG  
AATCCAGTGGAGGAAACCAAAGGAGAAGCATTCACTGATGGCAACATTGAAGTCAAATTG  
TGTGAACAGACAGAGGAGAAGAAAAGCTCAAACGACATCTGCTCTTAGGTCTGAA  
CTGGCTGAGAATTCTCCTTACAGACTGGCATTAA

TTATAACAATATCTTCATAATTAAATGCCCTTCAGATTGTAACCCAAAGAGAAAATTATT  
TGAGCAAAGGTCAAATACTCTTTTATTCTTAAGATGATGACAAGAAGAAAACAAATCAT  
GTTTCCATTAAAAAAATGACACGAGGCTAGTCAAAGTGCAGTGATGTTACAACCAATTGA  
TCACAATCATTAAACAGATTCTGTGTTCTCTCATTCCACTGCTTCACTTGACTAGC  
CTTAAAAAAAGCAACATGGAAGGCCAGGGCACGGTGGCTCATGCCTGTAATCCAGCACTT  
GGGAGGCTAGACGGCGGATCACGAGGTCAAGGAGATCAAACCATCCTGGCTAACACGG  
TGAAACCCCATCTGCTAAAATACAAAATTAGCGGGCGTGGTGGCGGGCACCTGTA  
GTCCCAGCTACTTGGGAGCCTCAGGGAGAATGGTGTGAACCCGGAGGGAGCTTG  
CACTGATCCGAGATCGTGCCTACTGCACCTGGCGAAAGAGCGAGACTCCCCGTC  
TCAAAAAAATTTTTGAAAATCGTAAACCATACTTTAAGATTATTCAGTGGATT  
TTTAAAATCTGTACAGAAATCAGGGTCTTAGCTAGCAGTTCTCCACGCAGTCA  
CTGTAATGTGACTATGTATTGCTAGATTGAATAAGAAAATAATATCTTCTCCTT  
GAAAAAAGGCCAGCTAGAGGATCCCT

## FIG. 6

MQALNITPEQFSRLLRDHNLTREQFIALYRLRPLVYTPELPGRAKLALVLTGVLIFALAL  
FGNALVFYVVTRSKAMRTVTNIFICSLALSDLITFFCIPVTMLQNIISDNWLGGAFICKM  
VPFVQSTAVVTEILTMTCIAVERHQGLVHPFKMKWQYTNRRAFTMLGVVWLAVAVIVGSPM  
WHVQQLEIKYDFLYEKEHICCLEEWTSVHQKIYTTFILVILFLLPLMVMLILYSKIGYE  
LWIKKRVGDGSVLRTIHGKEMSKIARKKKRAVIMMVTVVALFAVCWAPFHVVHMMIEYSN  
FEKEYDDVTIKMIFAIVQIIGFSNSICNPIVYAFMNEFKKNVLSAVCYCIVNKTFSQAQ  
RHGNSGITMMRKKAKFSLRENPVEETKGEAFSDGNIEVKLCEQTEEKKLKRHLALFRSE  
LAENSPLDSGH

**FIG. 7**

1 MQALNITPEQ FSRLLRDHNL TREQFIALYR LRPLVYTPEL PGRAKLALVL  
51 TGVLIFALAL FGNALVFYVV TRSKAMRTVT NIFICSLALS DLLITFFCIP  
101 VTMLQNIISDN WLGGAFICKM VPFVQSTAVV TEILTMTCIA VERHQGLVHP  
151 FKMKWQYTNR RAFTMLGVWW LVAVIVGSPM WHVQQLEIKY DFLYEKEHIC  
201 CLEEWTS PVH QKIYTTFILV ILFLLPLMVM LILYSKIGYE LWIKKRVGDG  
251 SVLRTIHGKE MSKIARKKKR AVIMMVTVVA LFAVCWAPFH VVHMMIEYSN  
301 FEKEYDDVTI KMIFAIQVII GFSNSICNPI VYAFMNENFK KNVLSAVCYC  
351 IVNKTFSPAQ RHGNSGITMM RKKAKFSLRE NPVEETKGEA FSDGNIEVKL  
401 CEQTEEKKKL KRHLALFRSE LAENSPLDSG H

Transmembrane Prediction for the Novel Human GPCR, HGPRBMY2

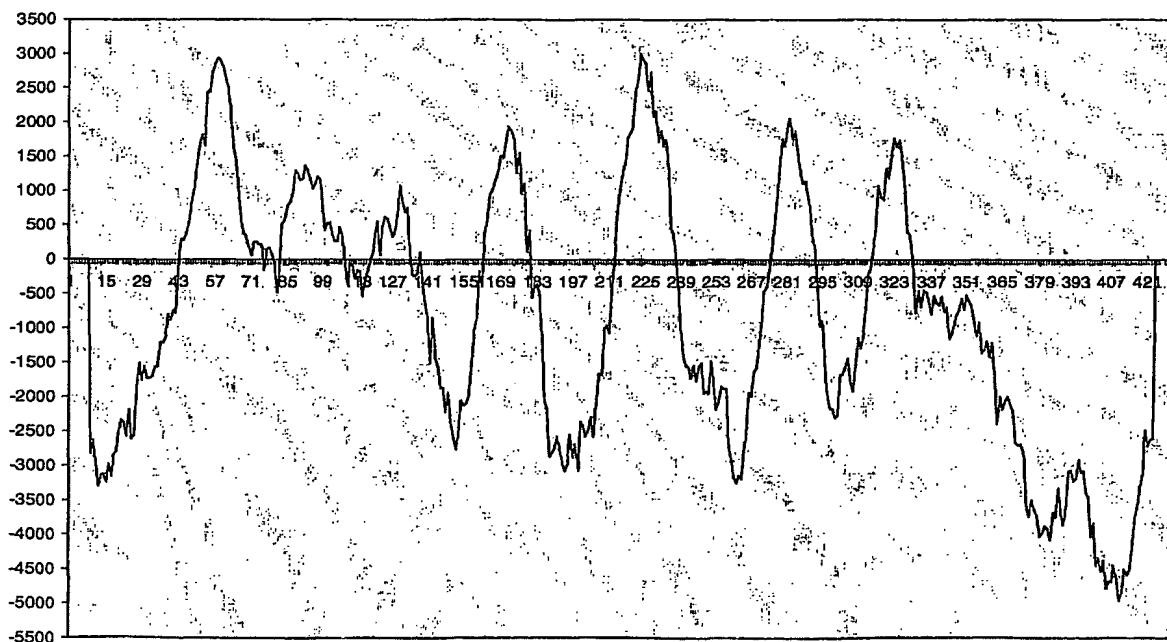


FIG. 8

|            |                                                                         |
|------------|-------------------------------------------------------------------------|
| OX2R_HUMAN | -----                                                                   |
| OX2R_RAT   | -----                                                                   |
| NY4R_MOUSE | -----                                                                   |
| NY4R_RAT   | -----                                                                   |
| NY6R_RABIT | -----                                                                   |
| Q9WVD0     | -----                                                                   |
| O57463     | -----                                                                   |
| NY2R_HUMAN | -----                                                                   |
| Q9Y5X5     | MNSFFGTPAASWCLLESDVSSAPDKEAGRERRALSVQQRGGPAWSGSLEWSRQSAGDRRR            |
| HGPRBMY2   | -----                                                                   |
| GALR_MOUSE | -----                                                                   |
| OX2R_HUMAN | -----MSGTKLEDSPPCRNWSSASEENET                                           |
| OX2R_RAT   | -----MSSTKLEDSLPRRNWSSASEENET                                           |
| NY4R_MOUSE | -----MNTSHFLAPFPG                                                       |
| NY4R_RAT   | -----MNTSHIMASPSA                                                       |
| NY6R_RABIT | -----MEVSELNDP                                                          |
| Q9WVD0     | -----MNSTSFSQLEN                                                        |
| O57463     | -----                                                                   |
| NY2R_HUMAN | -----MGPIGAEADENQTVEEMKVEQYGPQTTPRGEL                                   |
| Q9Y5X5     | LGLSRQTAKSWSRSRDRCCRAAWILVPAADRARRERFIMNEKWDTNSSENWHPIWN                |
| HGPRBMY2   | -----MQALNITPEQFSRLLR                                                   |
| GALR_MOUSE | -----MELA                                                               |
| OX2R_HUMAN | QEPFLNP[D]DYDDEELRMLWREYLHPKEYEWLIA[GYIT]V[EV]VALIGNV.LVCVAW.W.K        |
| OX2R_RAT   | QEPFLNP[D]DYDDEELRMLWREYLHPKEYEWLIA[GYIT]V[EV]VALIGNV.LVCVAW.W.K        |
| NY4R_MOUSE | SLQGKNGINPLDSEY.NH.SDGCQDSAELLAFIITTYSTETIILGVLGNICL.FVTTTROK           |
| NY4R_RAT   | FLQGKNGINPLDSIY.NL.SDGCQDSAELLAFIITTYSTETIILGVLGNICL.FVTTTROK           |
| NY6R_RABIT | ASNKTSAKSNSSAFY.YEES..CQSPSLALIILITAYTVLIMCICENLSELITIIFKKQR            |
| Q9WVD0     | HSVHYNLSEEKPSF..AFENDDCHLPLAVIFTLALAYGAMIIIILGVSGNLALLIIII.KOK          |
| O57463     | -----MER9HNNNSSW.LLEDPTCPASLSSTTFLLIVAYSTMLAVGLGCNTCLVVVLL.TROK         |
| NY2R_HUMAN | VPD.PEPELIDSTKL.I.....EVQVMLILAYCSIIILGVIGN.SLVITVMIKFK                 |
| Q9Y5X5     | VND.TKHHLYSDINI.T[V]VNYYLHQ.PQVAATIILISYFELIFFLICMCN.TVVCFLVMRNK        |
| HGPRBMY2   | DHNLTREQFIALYRL.RPLVYTPELPGRAKLAIVLTGVLI[F]ALALFGNIALVFTVTRSK           |
| GALR_MOUSE | MVNLSEGNGSDPEPP..APESRPLFGIGVENITLWVFGLT[F]AMGVGN.SLVITVJARSK           |
| OX2R_HUMAN | NHHMRIVTNYFIVNLSIADVLVITITCIPATIVVDITETWFFGQS[LC]KVI[PY]IOTIVSVS        |
| OX2R_RAT   | NHHMRIVTNYFIVNLSIADVLVITITCIPATIVVDITETWFFGQS[LC]KVI[PY]IOTIVSVS        |
| NY4R_MOUSE | ..EKSNTVTNLLIANLA[FSDF]LMCLICOP[ATV]TYT[IMD]WIFGEV[LC]KMIITFI[OCMSV]IVS |
| NY4R_RAT   | ..EKSNTVTNLLIANLA[FSDF]LMCLICOP[ATV]TYT[IMD]WIFGEV[LC]KMIITFI[OCMSV]IVS |
| NY6R_RABIT | ..EAQNVTNLLIANLSISDIL[LVCMC]IPFTATYT[IMD]WIFGEV[LC]KMIITFI[OCMSV]IVS    |
| Q9WVD0     | ..EMRNVTNLLIVNLSFSDILLVVATMCLPFTATYT[IMD]WIFGEV[LC]KMIITFI[OCMSV]IVS    |
| O57463     | ..EMRNVTNLLIVNLSFSDILLVVATMCLPFTATYT[IMD]WIFGEV[LC]KMIITFI[OCMSV]IVS    |
| NY2R_HUMAN | ..SMRIVTNFFIANLA[VAD]LLVNT[CEPFT]TYT[IM]GEWKMGPVLQHILVPIA[Q]LAVQVS      |
| Q9Y5X5     | ..HMHTVTNLFILIANLA[SDLLV]GTCMP[IT]L[EDN]IILAGWPFGNTMCKISGLVOG[ISVAAS    |
| HGPRBMY2   | ..AMRTVTNIFICSLAL[SDLLIT]FECI[PV]MLQNIISDNWLGGAFICKMVP[V]OSTAVVTE       |
| GALR_MOUSE | PGKPRSTT[ML]IADL[AYI]F[C]IPFQATVYAIPTWV[GA]FICKP[LM]FIFTVSMI[VS]        |

FIG. 9

|            |                                                           |                                            |                                      |    |
|------------|-----------------------------------------------------------|--------------------------------------------|--------------------------------------|----|
| OX2R_HUMAN | VLTLSCLIALDRWYAICHPLM..                                   | FKSTAKRARNSTVVIWIVSCIIIMI                  | QAIIVMECSTVFP                        | PG |
| OX2R_RAT   | VLTLSCLIALDRWYAICHPLM..                                   | FKSTAKRARNSTVVIWIVSCIIIMI                  | QAIIVMERSSMLPG                       |    |
| NY4R_MOUSE | ILSLVLVALERHOLIINP..                                      | TGWKPSIFQAYLGIVVIWPI                       | SCFLSLPFLANSTLNDLFHY                 |    |
| NY4R_RAT   | ILSLVLVALERHOLIENP..                                      | TGWKPSISQAYLGIVVIWPI                       | SCFLSLPFLANSILNDLFHY                 |    |
| NY6R_RABIT | IESLVLVALERHOLIENP..                                      | RGWKPSASHAYWCHMLIWIF                       | SLIISIPLIISYHLDPEPR                  |    |
| Q9WVD0     | IFSELVILIAVERHOLIINP..                                    | RGWRPNNRHAYIGIAVIWVIAVASSLPFM              | YQVLTDPEPQ                           |    |
| O57463     | IESMVLIALERHOLIHP..                                       | TGWNWPNVVRHSWVAVAVI                        | WIIACFESTPFLSFNILTNSPH               |    |
| NY2R_HUMAN | TYTITWIALDRHRCIVYHLESK..                                  | IISKRISELTIGIAGWGSALLASPLATF               | ....REVS                             |    |
| Q9Y5X5     | VEFELVAVIAVDRFOCVVYPPFKPK..                               | LTIIKTAEVVIMIWIWIAITIMSP                   | SAVMLHVQEKKYY                        |    |
| HGPRBMY2   | ELMTMTCIAVERHOGEVHPFKMKWQYINRRAFTMIGVWVAVIVGSP..          | MWHVQOLE..                                 |                                      |    |
| GALR_MOUSE | IFPLAAMSVDYRVAVHSRRSSSLRVSKNAILG                          | YGVFIWASIAMASPVAYHQRL..                    | EH                                   |    |
| OX2R_HUMAN | LANKTTLE.....                                             | TVCDERWGGE.....                            | YPKMYHICFFIVYMAPCLMVIAYLQIERRKL      |    |
| OX2R_RAT   | LANKTTLE.....                                             | TVCDERWGGE.....                            | YVPKMYHICFFIVYMAPCLMVIAYLQIERRKL     |    |
| NY4R_MOUSE | NHSKVVEFLEDKIV..                                          | CFVSNSSSPHHHLIYTTFI..                      | LLFOYCIPLAFFILVCYIRIYQRL             |    |
| NY4R_RAT   | NHSKVVEFLEDKIV..                                          | CFVSNSSSPHHHLIYTTFI..                      | LLFOYCIPLAFFILVCYIRIYQRL             |    |
| NY6R_RABIT | NEISLPTDLYSHHW..                                          | CVEHWPSKTNOLLYSTSL..                       | TMLOQFVPLGFMFICYLIKIVICL             |    |
| Q9WVD0     | NYTL..DAFKDKLIV..                                         | CFDQFPSDHSILSYTILL..                       | LVIQVFGPLCFIFTCYEKIVYIRL             |    |
| O57463     | NEISLPPNPFSDHFT..                                         | CIEQWPSEGNRLLTYTTL..                       | LLCQYCLPLAFLILVCYFRIELRD             |    |
| NY2R_HUMAN | LDEIIPDRE..                                               | IVACTEKWPGE..                              | ESIYGTIVYSLSSLLILVPLIG..ISFSYTRIWSKL |    |
| Q9Y5X5     | RFLRNSQNKTSPWYWCEDWPNOEMPKIYTTFI..                        | IYLAPELIVIDMYGRIGISL                       |                                      |    |
| HGPRBMY2   | TKYDFLMEKEHT..CCL                                         | EEWTSPVHQKILYTTFI..                        | LVIQVFGPLMVLILYSKIGYEL               |    |
| GALR_MOUSE | R.....                                                    | DSNQTFCWEQWPKNLHKKAY..                     | VVCTFMFGYLLPLIEICFCYAKVLNHL          |    |
| OX2R_HUMAN | WCQIPGTSSAWQRWKPLQPVSQPRGPGQPTKSRMSAVAAEIKQIRARRKTARMLMVV |                                            |                                      |    |
| OX2R_RAT   | WCQIPGTSSAWQRWKQPPVSQPRGSGQQSKARISAVAAEIKQIRARRKTARMLMVV  |                                            |                                      |    |
| NY4R_MOUSE | QEQK.....HMF.HA.....                                      | HACS..SRAG.....                            | QM..KRINSMLMTW                       |    |
| NY4R_RAT   | QQR.....RAF.HT.....                                       | HTCS..SRAG.....                            | QM..KRENGMLMAMV                      |    |
| NY6R_RABIT | HTRN.....SKEDRR.....                                      | RENE..SRAG.....                            | E..NKRINTMLLISIV                     |    |
| Q9WVD0     | KRRN.....NMMDKM.....                                      | RDSK..YRSS.....                            | E..SKRINIMLLISIV                     |    |
| O57463     | SRRK.....DAAVERA.....                                     | RGGR..QKKA.....                            | KGSKRVNAMLASTIV                      |    |
| NY2R_HUMAN | KNHVSP..GAA.....                                          | N..DHYH.....                               | ORROKTTKMLVCVV                       |    |
| Q9Y5X5     | FRAAVPHTGRK.....                                          | NQEQWHVIVS.....                            | RKKOKTIKMLLITVA                      |    |
| HGPRBMY2   | WIKKRVGDGSERTI.....                                       | HGKEMSKTA.....                             | KKKKRAVIMMVIVV                       |    |
| GALR_MOUSE | HKKLK.....                                                | NMSKKSEAS.....                             | K..KKTAQTVLVVV                       |    |
| OX2R_HUMAN | IVFAICVLPISLNLVVKRVMGMFAHTEDRETIVYAWFTFS..                | HWIVYANSAANPIIYNFLSG                       |                                      |    |
| OX2R_RAT   | IVFAICVLPISLNLVVKRVMGMFTHTEDRETIVYAWFTFS..                | HWIVYANSAANPIIYNFLSG                       |                                      |    |
| NY4R_MOUSE | TAFAVLWLPLHVFNLTLEDWYQ..                                  | EATPACHGNLIELM..                           | CHIILAMASTCVNPFIYGF                  |    |
| NY4R_RAT   | TAFAVLWLPLHVFNLTLEDWYQ..                                  | EATPACHGNLIELM..                           | CHIIFAMASTCVNPFIYGF                  |    |
| NY6R_RABIT | VIIFAACCWLPLNTFNLTFDWYH..                                 | EIVIIMSCHHDLVFAI..                         | CHIEVAMVSTCINPLFYGF                  |    |
| Q9WVD0     | VAFAVCWLPLNTFNLTFDWNH..                                   | QIATATCNHNLLFELI..                         | CHIETAMISTCVNPFIYGF                  |    |
| O57463     | AAFAICWLPLNTFNLTFDWNH..                                   | EATPVCQHDAIFSA..                           | CHIETAMISTCVNPFIYGF                  |    |
| NY2R_HUMAN | VVFAVSWLPLHAFQAVD..IDSQVLDIKEYKL..IIFTV..FHIIAMCSTF       | ANPLIYGMNS                                 |                                      |    |
| Q9Y5X5     | LLFILISWLPLWTLM..ISDYLADLSPNE..QIINI..YIVPF..AHWIAF       | GNSSVNPIIYGF                               |                                      |    |
| HGPRBMY2   | ALFAVCWAIFHIVHMMIEYSNFE..KEYDDVTIKMIFAG..VOLIGFSNSIC      | CNPPIIVYAFMNE                              |                                      |    |
| GALR_MOUSE | VVEGISWLPHIVVH..                                          | WAEFG..AFPLTPASFFERIATACLAYSNSSVNPIIYAFLSE |                                      |    |

FIG. 9 Continued

|            |                                                                                                                                                                    |
|------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| OX2R_HUMAN | KEREEFKAAFS <del>CCCLGVHHRQE</del> .. <del>DRI</del> TRGR <del>T</del> TESRK <del>SLTTQIS</del> <del>NFDNISKLSEQVVL</del> T                                        |
| OX2R_RAT   | KEREEFKAAFS.CCLGVHRRQG.. <del>DRI</del> ARG <del>R</del> TESRK <del>SLTTQIS</del> <del>NFDNISKLSEH</del> VALT                                                      |
| NY4R_MOUSE | NEKKNDIKALVLTCHC.RSPQGES.EH <del>PL</del> STV <del>HT</del> LSKG <del>SRM</del> GS <del>KS</del> SNFI~~~~~                                                         |
| NY4R_RAT   | NEKKDTIKALVLT <del>RC</del> R.PPQGEP.BP <del>PL</del> STV <del>HT</del> LSKG <del>SRM</del> GS <del>KS</del> SNVM~~~~~                                             |
| NY6R_RABIT | NEQKDIIVVLIHHCLC.FALR.ERYEN <del>MA</del> IST <del>HT</del> DESKGST...RVAHIPAGI~~~~~                                                                               |
| Q9WVD0     | NEORDL <del>OFF</del> FNFC.D.FRS <del>HDD</del> YET <del>TEAM</del> S <del>IMH</del> TDVSKTST...KOAS.PLAFKKI <del>SC</del> VE                                      |
| O57463     | NEOKNLRS <del>LL</del> SR <del>RC</del> C.WG.PAESY <del>ES</del> FP <del>LS</del> AN <del>ST</del> GITKG <del>SI</del> LSNGSASTYQPHKKNSLEQ                         |
| NY2R_HUMAN | NY <del>KA</del> FLSAFR...C..EQ <del>IL</del> DAI..HSEV <del>W</del> ..FKA <del>KK</del> EV <del>R</del> KNSGP <del>NDS</del> FTEATNV~                             |
| Q9Y5X5     | NE <del>REG</del> FQEA <del>Q</del> LOLC..QKRAKPM..E...AYE..LKA <del>KSH</del> YLI..NTS.NQLVQESTFQN                                                                |
| HGPRBMY2   | NE <del>KK</del> N <del>Y</del> LSAVCYCIV..NKT <del>FS</del> PAQRHGN <del>SC</del> HM <del>MR</del> KKAKF <del>SL</del> REN..PVEETKGEAFSD                          |
| GALR_MOUSE | NE <del>KK</del> Y <del>AK</del> QVFK <del>CH</del> V <del>C</del> DESPR <del>SET</del> KEN <del>KS</del> RMD <del>TP</del> PS <del>T</del> N <del>CH</del> Y~~~~~ |
| OX2R_HUMAN | SISTLPAANGAGPLQN <del>W</del> ~~~~~                                                                                                                                |
| OX2R_RAT   | SISTLPAANGAGPLQN <del>W</del> Y <del>QQGV</del> P <del>SS</del> LL <del>ST</del> W <del>LEV</del> ~~~~~                                                            |
| NY4R_MOUSE | ~~~~~                                                                                                                                                              |
| NY4R_RAT   | ~~~~~                                                                                                                                                              |
| NY6R_RABIT | ~~~~~                                                                                                                                                              |
| Q9WVD0     | NEKI~~~~~                                                                                                                                                          |
| O57463     | KESI~~~~~                                                                                                                                                          |
| NY2R_HUMAN | ~~~~~                                                                                                                                                              |
| Q9Y5X5     | PHGETLLYRKSAEKPQQELVMEELK.ETTN <del>SSEI</del> ~~~~~                                                                                                               |
| HGPRBMY2   | GNIEVKLCEQTE <del>KK</del> KLKRHL <del>AL</del> FR <del>SEL</del> A <del>EN</del> SPLDSGH                                                                          |
| GALR_MOUSE | ~~~~~                                                                                                                                                              |

FIG. 9 Continued

FIG. 10

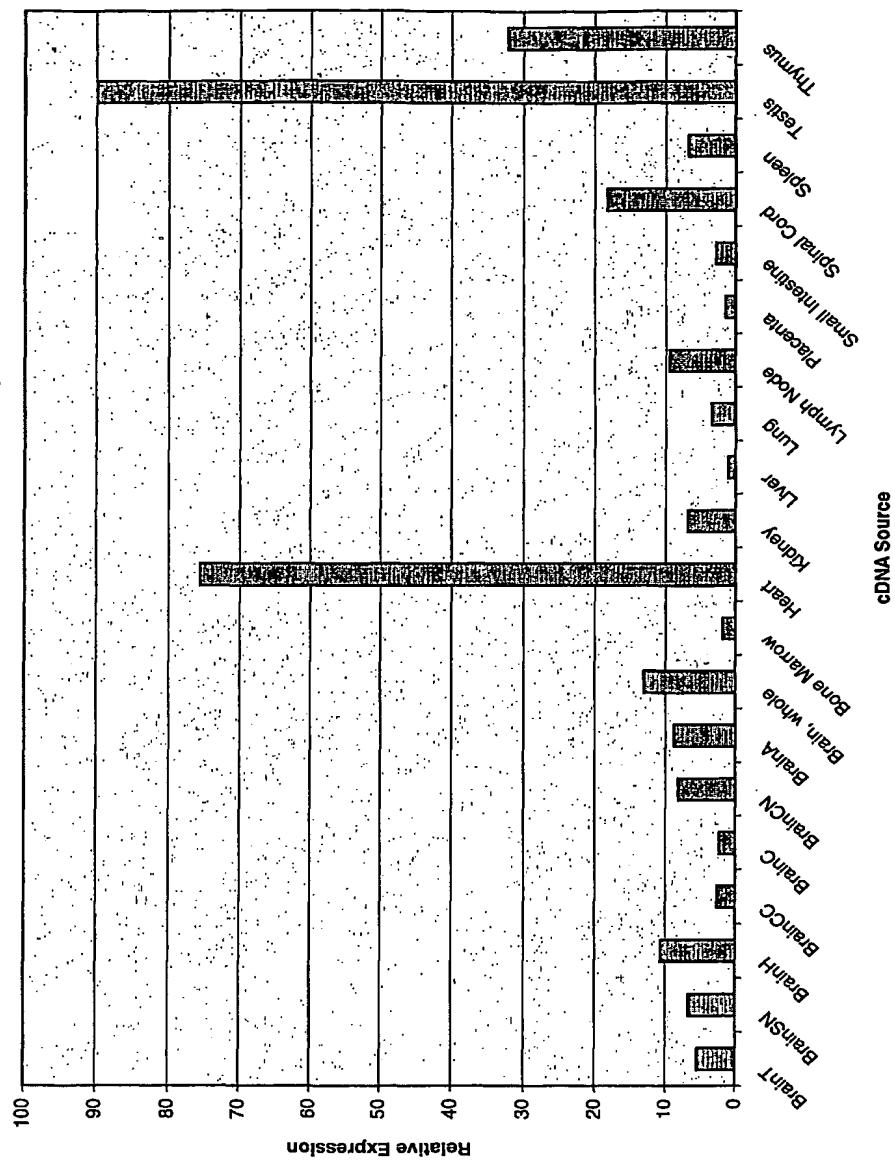


Figure 11

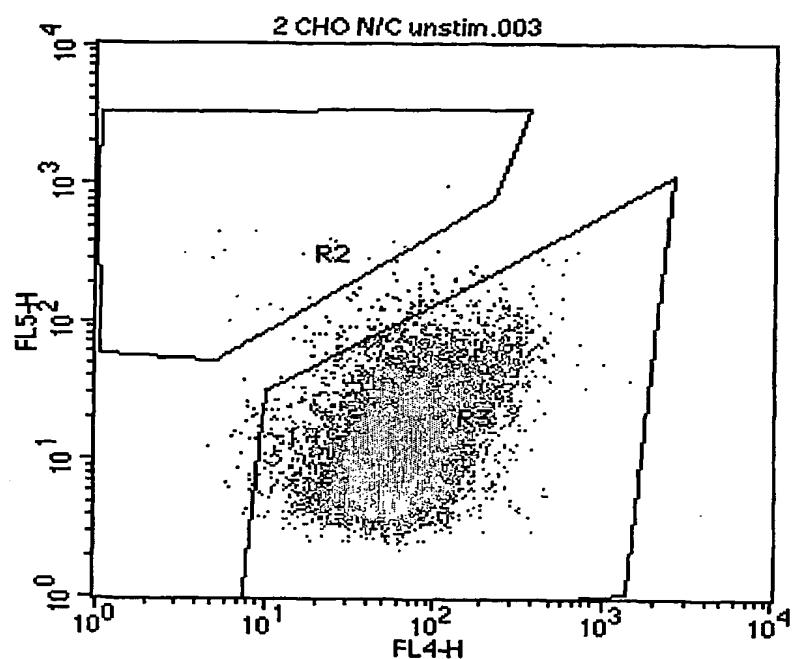


Figure 12

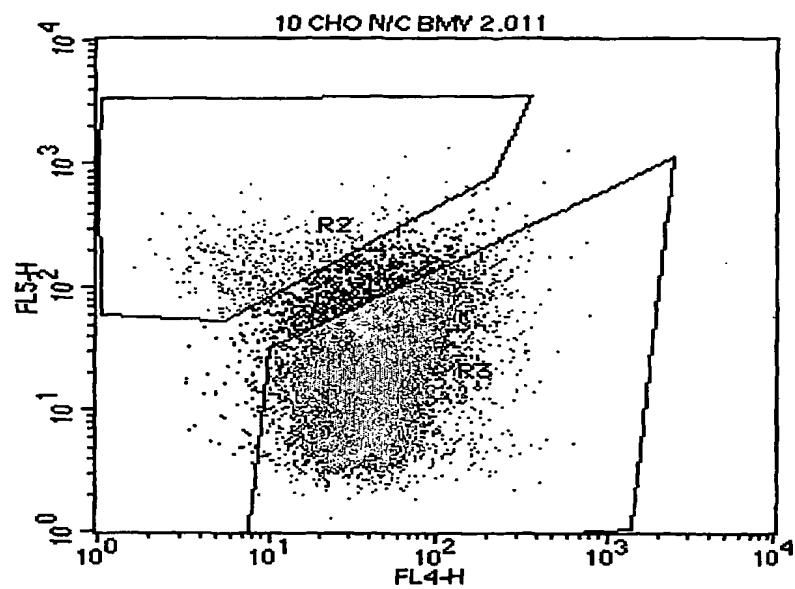


Figure 13

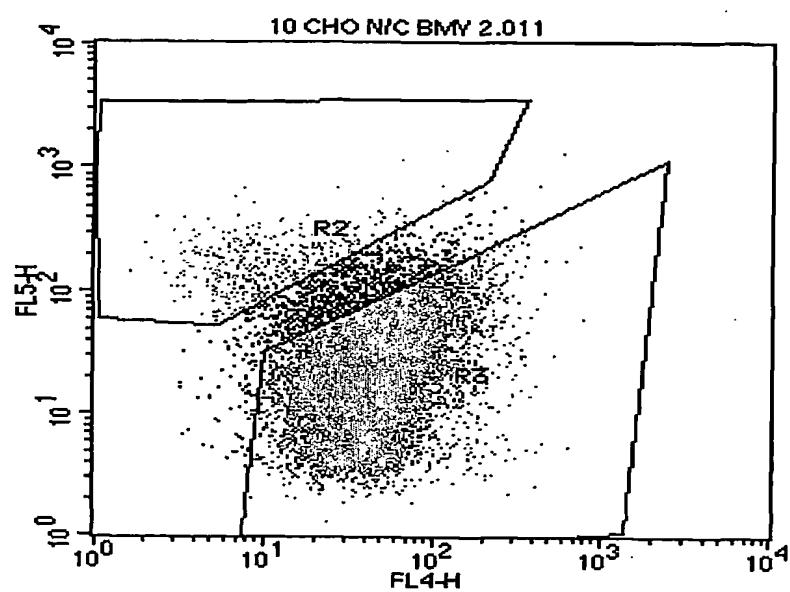
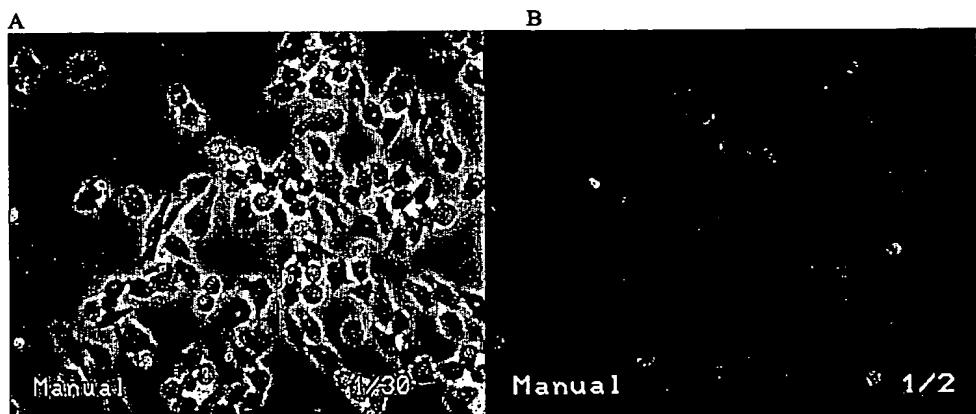
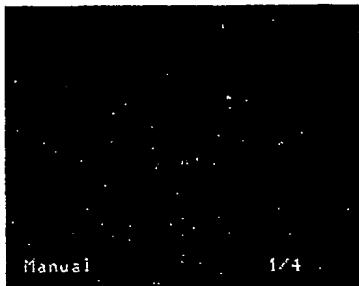


Figure 14



**Figure 15**

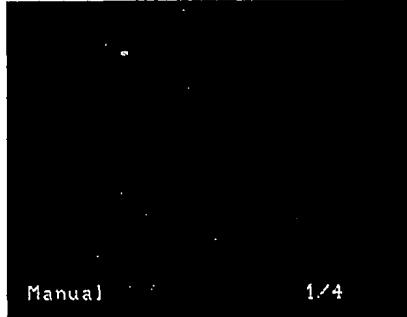
a. Cho-NFAT CRE



b. Cho-NFAT CRE + F/T/P



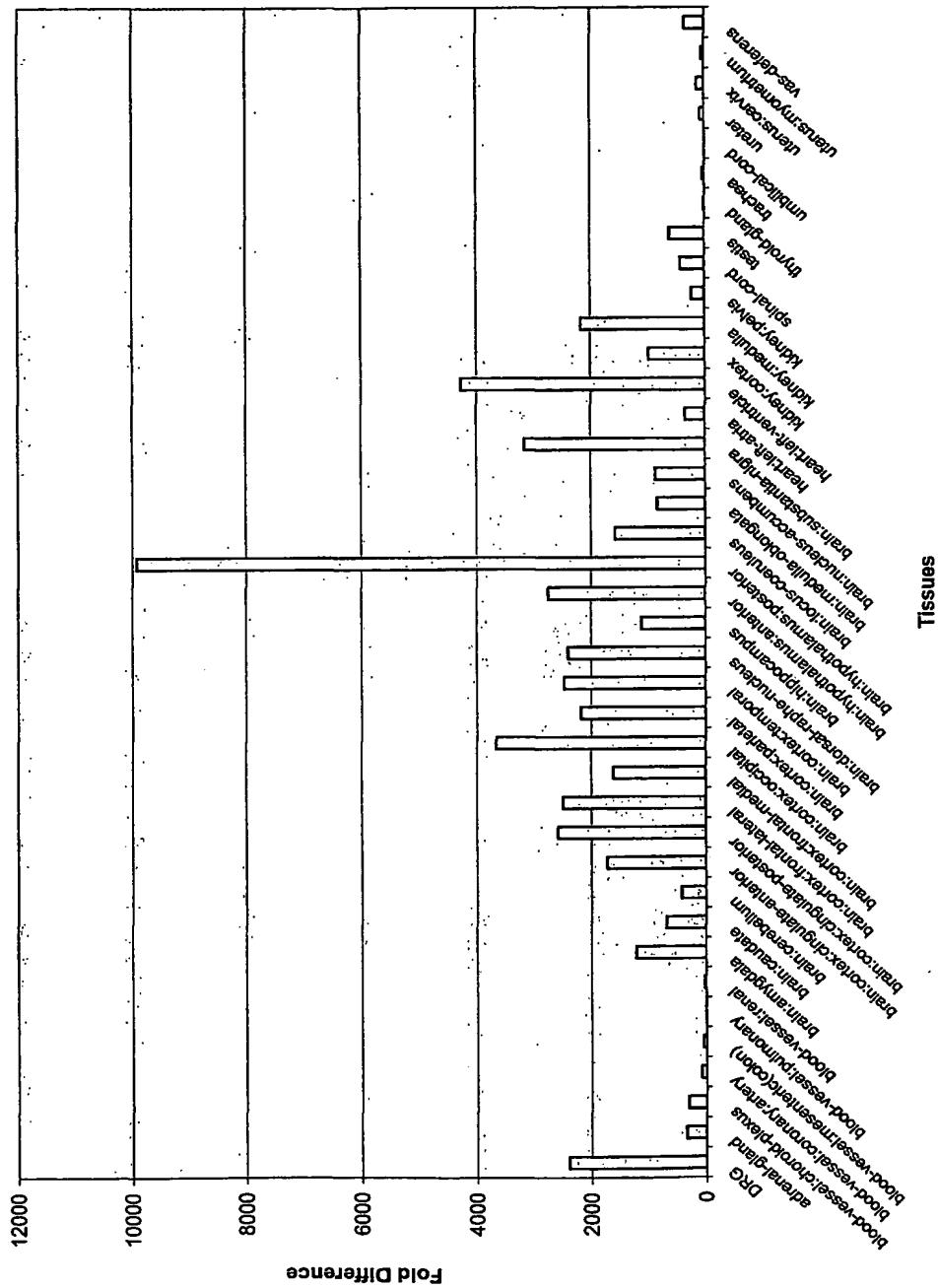
c. Cho-NFAT CRE HGPRBMY2 Intermediate



d. Cho-NFAT CRE HGPRBMY2 High



Figure 16



## SEQUENCE LISTING

<110> Bristol-Myers Squibb Company

<120> G-PROTEIN COUPLED RECEPTOR NUCLEIC ACIDS, POLYPEPTIDES, ANTIBODIES AND USES THEREOF

<130> D0132 PCT

<150> US 60/270,793  
<151> 2001-02-23

<150> US 60/270,792  
<151> 2001-02-23

<150> US 60/296,427  
<151> 2001-06-06

<160> 58

<170> PatentIn version 3.0

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<220>  
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|                                                                     |  |     |
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| ggccgcctt gcaagggtgc tggacagatg gaactggaaag ggcagccgtc tgccgcccac   |  | 60  |
| gaacaccttc tcaagcactt tgagtgacca cggcttgcaa gctgggtggct ggccccccgaa |  | 120 |
| gtcccggtct ctgaggcacg gccgtcgact taagcgttgc atcctgttac ctggagaccc   |  | 180 |
| tctgagctct cacctgctac ttctgcccgt gcttctgcac agagccccgg cgaggacccc   |  | 240 |
| tccagg atg cag gtc ccg aac agc acc ggc ccg gac aac ggc acg ctg      |  | 288 |
| Met Gln Val Pro Asn Ser Thr Gly Pro Asp Asn Ala Thr Leu             |  |     |
| 1                   5                   10                          |  |     |
| cag atg ctg ccg aac ccg gcg atc gcg gtg gcc ctg ccc gtg gtg tac     |  | 336 |
| Gln Met Leu Arg Asn Pro Ala Ile Ala Val Ala Leu Pro Val Val Tyr     |  |     |
| 15                   20                   25                   30   |  |     |
| tcg ctg gtg gcg gcg gtc agc atc ccg ggc aac ctc ttc tct ctg tgg     |  | 384 |
| Ser Leu Val Ala Ala Val Ser Ile Pro Gly Asn Leu Phe Ser Leu Trp     |  |     |
| 35                   40                   45                        |  |     |
| gtg ctg tgc ccg ccg atg ggg ccc aga tcc ccg tcg gtc atc ttc atg     |  | 432 |
| Val Leu Cys Arg Arg Met Gly Pro Arg Ser Pro Ser Val Ile Phe Met     |  |     |
| 50                   55                   60                        |  |     |
| atc aac ctg agc gtc acg gac ctg atg ctg gcc agc gtg ttg cct ttc     |  | 480 |

|                                                                 |     |     |      |
|-----------------------------------------------------------------|-----|-----|------|
| Ile Asn Leu Ser Val Thr Asp Leu Met Leu Ala Ser Val Leu Pro Phe |     |     |      |
| 65                                                              | 70  | 75  |      |
| caa atc tac tac cat tgc aac cgc cac cac tgg gta ttc ggg gtg ctg |     |     | 528  |
| Gln Ile Tyr Tyr His Cys Asn Arg His His Trp Val Phe Gly Val Leu |     |     |      |
| 80                                                              | 85  | 90  |      |
| ctt tgc aac gtg gtg acc gtg gcc ttt tac gca aac atg tat tcc agc |     |     | 576  |
| Leu Cys Asn Val Val Thr Val Ala Phe Tyr Ala Asn Met Tyr Ser Ser |     |     |      |
| 95                                                              | 100 | 105 | 110  |
| atc ctc acc atg acc tgt atc agc gtg gag cgc ttc ctg ggg gtc ctg |     |     | 624  |
| Ile Leu Thr Met Thr Cys Ile Ser Val Glu Arg Phe Leu Gly Val Leu |     |     |      |
| 115                                                             | 120 | 125 |      |
| tac ccg ctc agc tcc aag cgc tgg cgc cgc cgt cgt tac gcg gtg gcc |     |     | 672  |
| Tyr Pro Leu Ser Ser Lys Arg Trp Arg Arg Arg Tyr Ala Val Ala     |     |     |      |
| 130                                                             | 135 | 140 |      |
| gcg tgt gca ggg acc tgg ctg ctg ctc ctg acc gcc ctg tcc ccg ctg |     |     | 720  |
| Ala Cys Ala Gly Thr Trp Leu Leu Leu Thr Ala Leu Ser Pro Leu     |     |     |      |
| 145                                                             | 150 | 155 |      |
| gcg cgc acc gat ctc acc tac ccg gtg cac gcc ctg ggc atc atc acc |     |     | 768  |
| Ala Arg Thr Asp Leu Thr Tyr Pro Val His Ala Leu Gly Ile Ile Thr |     |     |      |
| 160                                                             | 165 | 170 |      |
| tgc ttc gac gtc ctc aag tgg acg atg ctc ccc agc gtg gcc atg tgg |     |     | 816  |
| Cys Phe Asp Val Leu Lys Trp Thr Met Leu Pro Ser Val Ala Met Trp |     |     |      |
| 175                                                             | 180 | 185 | 190  |
| gcc gtg ttc ctc ttc acc atc ttc atc ctg ctg ttc ctc atc ccg ttc |     |     | 864  |
| Ala Val Phe Leu Phe Thr Ile Phe Ile Leu Leu Phe Leu Ile Pro Phe |     |     |      |
| 195                                                             | 200 | 205 |      |
| gtg atc acc gtg gct tgt tac acg gcc acc atc ctc aag ctg ttg cgc |     |     | 912  |
| Val Ile Thr Val Ala Cys Tyr Thr Ala Thr Ile Leu Lys Leu Leu Arg |     |     |      |
| 210                                                             | 215 | 220 |      |
| acg gag gag gcg cac ggc cgg gag cag cgg agg cgc gcg gtg ggc ctg |     |     | 960  |
| Thr Glu Glu Ala His Gly Arg Glu Gln Arg Arg Arg Ala Val Gly Leu |     |     |      |
| 225                                                             | 230 | 235 |      |
| gcc gcg gtg gtc ttg ctg gcc ttt gtc acc tgc ttc gcc ccc aac aac |     |     | 1008 |
| Ala Ala Val Val Leu Leu Ala Phe Val Thr Cys Phe Ala Pro Asn Asn |     |     |      |
| 240                                                             | 245 | 250 |      |
| ttc gtg ctc ctg gcg cac atc gtg agc cgc ctg ttc tac ggc aag agc |     |     | 1056 |
| Phe Val Leu Leu Ala His Ile Val Ser Arg Leu Phe Tyr Gly Lys Ser |     |     |      |
| 255                                                             | 260 | 265 | 270  |
| tac tac cac gtg tac aag ctc acg ctg tgt ctc agc tgc ctc aac aac |     |     | 1104 |
| Tyr Tyr His Val Tyr Lys Leu Thr Leu Cys Leu Ser Cys Leu Asn Asn |     |     |      |
| 275                                                             | 280 | 285 |      |
| tgt ctg gac ccg ttt gtt tat tac ttt gcg tcc cgg gaa ttc cag ctg |     |     | 1152 |
| Cys Leu Asp Pro Phe Val Tyr Phe Ala Ser Arg Glu Phe Gln Leu     |     |     |      |

| 290                                                                                                                                                                                                                                              | 295 | 300 |                              |
|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----|-----|------------------------------|
| cgc ctg cgg gaa tat ttg ggc tgc cgc cgg gtg ccc aga gac acc ctg<br>Arg Leu Arg Glu Tyr Leu Gly Cys Arg Arg Val Pro Arg Asp Thr Leu<br>305                                                                                                        | 310 | 315 | 1200                         |
| gac acg cgc cgc gag agc ctc ttc tcc gcc agg acc acg tcc gtg cgc<br>Asp Thr Arg Arg Glu Ser Leu Phe Ser Ala Arg Thr Thr Ser Val Arg<br>320                                                                                                        | 325 | 330 | 1248                         |
| tcc gag gcc ggt gcg cac cct gaa ggg atg gag gga gcc acc agg ccc<br>Ser Glu Ala Gly Ala His Pro Glu Gly Met Glu Gly Ala Thr Arg Pro<br>335                                                                                                        | 340 | 345 | 1296                         |
| ggc ctc cag agg cag gag agt gtg ttc tccctgtcga catcgccct<br>Gly Leu Gln Arg Gln Glu Ser Val Phe<br>355                                                                                                                                           |     |     | 1343                         |
| tagttgtgg tctggccttc tccattctcc tccaggggtt ctggctccg tagcccggtg<br>cacgcccggaaa tttctgttta tttcactcag gggcactgtg gttgctgtgg ttggattct<br>tcttcagag gagcgcctgg ggctcctgca agtcagctac tctccgtgcc cacttccct<br>cacacacaca ccccccctcg t gccgaattct t |     |     | 1403<br>1463<br>1523<br>1554 |
| <p>&lt;210&gt; 2<br/> &lt;211&gt; 359<br/> &lt;212&gt; PRT<br/> &lt;213&gt; Homo sapiens</p> <p>&lt;400&gt; 2</p>                                                                                                                                |     |     |                              |
| Met Gln Val Pro Asn Ser Thr Gly Pro Asp Asn Ala Thr Leu Gln Met<br>1                                                                                                                                                                             | 5   | 10  | 15                           |
| Leu Arg Asn Pro Ala Ile Ala Val Ala Leu Pro Val Val Tyr Ser Leu<br>20                                                                                                                                                                            | 25  | 30  |                              |
| Val Ala Ala Val Ser Ile Pro Gly Asn Leu Phe Ser Leu Trp Val Leu<br>35                                                                                                                                                                            | 40  | 45  |                              |
| Cys Arg Arg Met Gly Pro Arg Ser Pro Ser Val Ile Phe Met Ile Asn<br>50                                                                                                                                                                            | 55  | 60  |                              |
| Leu Ser Val Thr Asp Leu Met Leu Ala Ser Val Leu Pro Phe Gln Ile<br>65                                                                                                                                                                            | 70  | 75  | 80                           |
| Tyr Tyr His Cys Asn Arg His His Trp Val Phe Gly Val Leu Leu Cys<br>85                                                                                                                                                                            | 90  |     | 95                           |

Asn Val Val Thr Val Ala Phe Tyr Ala Asn Met Tyr Ser Ser Ile Leu  
100 105 110

Thr Met Thr Cys Ile Ser Val Glu Arg Phe Leu Gly Val Leu Tyr Pro  
115 120 125

Leu Ser Ser Lys Arg Trp Arg Arg Arg Tyr Ala Val Ala Ala Cys  
130 135 140

Ala Gly Thr Trp Leu Leu Leu Thr Ala Leu Ser Pro Leu Ala Arg  
145 150 155 160

Thr Asp Leu Thr Tyr Pro Val His Ala Leu Gly Ile Ile Thr Cys Phe  
165 170 175

Asp Val Leu Lys Trp Thr Met Leu Pro Ser Val Ala Met Trp Ala Val  
180 185 190

Phe Leu Phe Thr Ile Phe Ile Leu Leu Phe Leu Ile Pro Phe Val Ile  
195 200 205

Thr Val Ala Cys Tyr Thr Ala Thr Ile Leu Lys Leu Leu Arg Thr Glu  
210 215 220

Glu Ala His Gly Arg Glu Gln Arg Arg Ala Val Gly Leu Ala Ala  
225 230 235 240

Val Val Leu Ala Phe Val Thr Cys Phe Ala Pro Asn Asn Phe Val  
245 250 255

Leu Leu Ala His Ile Val Ser Arg Leu Phe Tyr Gly Lys Ser Tyr Tyr  
260 265 270

His Val Tyr Lys Leu Thr Leu Cys Leu Ser Cys Leu Asn Asn Cys Leu  
275 280 285

Asp Pro Phe Val Tyr Tyr Phe Ala Ser Arg Glu Phe Gln Leu Arg Leu  
290 295 300

Arg Glu Tyr Leu Gly Cys Arg Arg Val Pro Arg Asp Thr Leu Asp Thr  
305 310 315 320

Arg Arg Glu Ser Leu Phe Ser Ala Arg Thr Thr Ser Val Arg Ser Glu  
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Gln Arg Gln Glu Ser Val Phe  
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<400> 7

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25

<210> 8

<211> 25

<212> DNA

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<400> 8

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25

<210> 9

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<223> Synthesized Oligonucleotide.

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25

<210> 10

<211> 16

<212> DNA

<213> Homo sapiens

<400> 10

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16

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<400> 11

ggctcgcctc ttccatgtc

19

<210> 12

<211> 25

<212> DNA

<213> Homo sapiens

<400> 12

|                                                                    |     |
|--------------------------------------------------------------------|-----|
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| <210> 13                                                           |     |
| <211> 24                                                           |     |
| <212> DNA                                                          |     |
| <213> Homo sapiens                                                 |     |
| <400> 13                                                           |     |
| gaggatgagg agagctatga caca                                         | 24  |
| <210> 14                                                           |     |
| <211> 22                                                           |     |
| <212> DNA                                                          |     |
| <213> Homo sapiens                                                 |     |
| <400> 14                                                           |     |
| cccttgcac tcataacgtc ag                                            | 22  |
| <210> 15                                                           |     |
| <211> 29                                                           |     |
| <212> DNA                                                          |     |
| <213> Homo sapiens                                                 |     |
| <400> 15                                                           |     |
| aaacacacag tcatcatagg gcagctcg                                     | 29  |
| <210> 16                                                           |     |
| <211> 2448                                                         |     |
| <212> DNA                                                          |     |
| <213> Homo sapiens                                                 |     |
| <220>                                                              |     |
| <221> CDS                                                          |     |
| <222> (359)..(1651)                                                |     |
| <400> 16                                                           |     |
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| cctcgcccta gggaaagagca agggaaaac tttatggaa ccgcgaacat tttttggta    | 120 |
| ctgagatcga gtctcccagt gctttggctt cccgcctttt tatcttgggt ttgatccctg  | 180 |
| agctgctctc ctttccgaa cctccgggg tgcaagcctag agccctcccg cgccgtgac    | 240 |
| tccagagtag aggaagggag gcggccctccg gctggtcccc cgaagccctc gctgccccgc | 300 |
| agatgcggat gcccagccag tagcggggcg tgccccgcg tcccgggagc gcacagca     | 358 |
| atg cag gcg ctt aac att acc ccg gag cag ttc tct cgg ctg ctg cgg    | 406 |
| Met Gln Ala Asn Ile Thr Pro Glu Gln Phe Ser Arg Leu Leu Arg        |     |
| 1 5 10 15                                                          |     |
| gac cac aac ctg acg cgg gag cag ttc atc gct ctg tac cgg ctg cga    | 454 |

|                                                                 |     |     |      |
|-----------------------------------------------------------------|-----|-----|------|
| Asp His Asn Leu Thr Arg Glu Gln Phe Ile Ala Leu Tyr Arg Leu Arg |     |     |      |
| 20                                                              | 25  | 30  |      |
| ccg ctc gtc tac acc cca gag ctg ccg gga cgc gcc aag ctg gcc ctc |     |     | 502  |
| Pro Leu Val Tyr Thr Pro Glu Leu Pro Gly Arg Ala Lys Leu Ala Leu |     |     |      |
| 35                                                              | 40  | 45  |      |
| gtg ctc acc ggc gtg ctc atc ttc gcc ctg gcg ctc ttt ggc aat gct |     |     | 550  |
| Val Leu Thr Gly Val Leu Ile Phe Ala Leu Ala Leu Phe Gly Asn Ala |     |     |      |
| 50                                                              | 55  | 60  |      |
| ctg gtg ttc tac gtg gtg acc cgc agc aag gcc atg cgc acc gtc acc |     |     | 598  |
| Leu Val Phe Tyr Val Val Thr Arg Ser Lys Ala Met Arg Thr Val Thr |     |     |      |
| 65                                                              | 70  | 75  | 80   |
| aac atc ttt atc tgc tcc ttg gcg ctc agt gac ctg ctc atc acc ttc |     |     | 646  |
| Asn Ile Phe Ile Cys Ser Leu Ala Leu Ser Asp Leu Leu Ile Thr Phe |     |     |      |
| 85                                                              | 90  | 95  |      |
| ttc tgc att ccc gtc acc atg ctc cag aac att tcc gac aac tgg ctg |     |     | 694  |
| Phe Cys Ile Pro Val Thr Met Leu Gln Asn Ile Ser Asp Asn Trp Leu |     |     |      |
| 100                                                             | 105 | 110 |      |
| ggg ggt gct ttc att tgc aag atg gtg cca ttt gtc cag tct acc gct |     |     | 742  |
| Gly Gly Ala Phe Ile Cys Lys Met Val Pro Phe Val Gln Ser Thr Ala |     |     |      |
| 115                                                             | 120 | 125 |      |
| gtt gtg aca gaa atc ctc act atg acc tgc att gct gtg gaa agg cac |     |     | 790  |
| Val Val Thr Glu Ile Leu Thr Met Thr Cys Ile Ala Val Glu Arg His |     |     |      |
| 130                                                             | 135 | 140 |      |
| cag gga ctt gtg cat cct ttt aaa atg aag tgg caa tac acc aac cga |     |     | 838  |
| Gln Gly Leu Val His Pro Phe Lys Met Lys Trp Gln Tyr Thr Asn Arg |     |     |      |
| 145                                                             | 150 | 155 | 160  |
| agg gct ttc aca atg cta ggt gtg gtc tgg ctg gtg gca gtc atc gta |     |     | 886  |
| Arg Ala Phe Thr Met Leu Gly Val Val Trp Leu Val Ala Val Ile Val |     |     |      |
| 165                                                             | 170 | 175 |      |
| gga tca ccc atg tgg cac gtg caa caa ctt gag atc aaa tat gac ttc |     |     | 934  |
| Gly Ser Pro Met Trp His Val Gln Gln Leu Glu Ile Lys Tyr Asp Phe |     |     |      |
| 180                                                             | 185 | 190 |      |
| cta tat gaa aag gaa cac atc tgc tgc tta gaa gag tgg acc agc cct |     |     | 982  |
| Leu Tyr Glu Lys Glu His Ile Cys Cys Leu Glu Glu Trp Thr Ser Pro |     |     |      |
| 195                                                             | 200 | 205 |      |
| gtg cac cag aag atc tac acc acc ttc atc ctt gtc atc ctc ttc ctc |     |     | 1030 |
| Val His Gln Lys Ile Tyr Thr Phe Ile Leu Val Ile Leu Phe Leu     |     |     |      |
| 210                                                             | 215 | 220 |      |
| ctg cct ctt atg gtg atg ctt att ctg tac agt aaa att ggt tat gaa |     |     | 1078 |
| Leu Pro Leu Met Val Met Leu Ile Leu Tyr Ser Lys Ile Gly Tyr Glu |     |     |      |
| 225                                                             | 230 | 235 | 240  |
| ctt tgg ata aag aaa aga gtt ggg gat ggt tca gtg ctt cga act att |     |     | 1126 |
| Leu Trp Ile Lys Lys Arg Val Gly Asp Gly Ser Val Leu Arg Thr Ile |     |     |      |

| 245                                                                                                                                       | 250 | 255 |      |
|-------------------------------------------------------------------------------------------------------------------------------------------|-----|-----|------|
| cat gga aaa gaa atg tcc aaa ata gcc agg aag aag aaa cga gct gtc<br>His Gly Lys Glu Met Ser Lys Ile Ala Arg Lys Lys Arg Ala Val<br>260     | 265 | 270 | 1174 |
| att atg atg gtg aca gtg gtg gct ctc ttt gct gtg tgc tgg gca cca<br>Ile Met Met Val Thr Val Val Ala Leu Phe Ala Val Cys Trp Ala Pro<br>275 | 280 | 285 | 1222 |
| ttc cat gtt gtc cat atg atg att gaa tac agt aat ttt gaa aag gaa<br>Phe His Val Val His Met Met Ile Glu Tyr Ser Asn Phe Glu Lys Glu<br>290 | 295 | 300 | 1270 |
| tat gat gat gtc aca atc aag atg att ttt gct atc gtg caa att att<br>Tyr Asp Asp Val Thr Ile Lys Met Ile Phe Ala Ile Val Gln Ile Ile<br>305 | 310 | 315 | 1318 |
| gga ttt tcc aac tcc atc tgt aat ccc att gtc tat gca ttt atg aat<br>Gly Phe Ser Asn Ser Ile Cys Asn Pro Ile Val Tyr Ala Phe Met Asn<br>325 | 330 | 335 | 1366 |
| gaa aac ttc aaa aaa aat gtt ttg tct gca gtt tgt tat tgc ata gta<br>Glu Asn Phe Lys Lys Asn Val Leu Ser Ala Val Cys Tyr Cys Ile Val<br>340 | 345 | 350 | 1414 |
| aat aaa acc ttc tct cca gca caa agg cat gga aat tca gga att aca<br>Asn Lys Thr Phe Ser Pro Ala Gln Arg His Gly Asn Ser Gly Ile Thr<br>355 | 360 | 365 | 1462 |
| atg atg cgg aag aaa gca aag ttt tcc ctc aga gag aat cca gtg gag<br>Met Met Arg Lys Lys Ala Lys Phe Ser Leu Arg Glu Asn Pro Val Glu<br>370 | 375 | 380 | 1510 |
| gaa acc aaa gga gaa gca ttc agt gat ggc aac att gaa gtc aaa ttg<br>Glu Thr Lys Gly Glu Ala Phe Ser Asp Gly Asn Ile Glu Val Lys Leu<br>385 | 390 | 395 | 1558 |
| tgt gaa cag aca gag gag aag aaa aag ctc aaa cga cat ctt gct ctc<br>Cys Glu Gln Thr Glu Glu Lys Lys Leu Lys Arg His Leu Ala Leu<br>405     | 410 | 415 | 1606 |
| ttt agg tct gaa ctg gct gag aat tct cct tta gac agt ggg cat<br>Phe Arg Ser Glu Leu Ala Glu Asn Ser Pro Leu Asp Ser Gly His<br>420         | 425 | 430 | 1651 |
| taattataac aatatcttca taattaatgc ccttcagatt gtaacccaaa gagaaaatta                                                                         |     |     | 1711 |
| ttttgagcaa aggtcaaata ctcttttat tcttaagatg atgacaagaa gaaaacaaat                                                                          |     |     | 1771 |
| catgtttcca ttaaaaaatg acacgaggct agtccaagtg cagtgatgtt tacaaccaat                                                                         |     |     | 1831 |
| tgatcacaat catttaacag atttctgtgt tccttctcat tcccactgct tcacttgact                                                                         |     |     | 1891 |
| agccttaaaa aagcaacatg gaaggccagg cacggtggt catgcctgta atcccagcac                                                                          |     |     | 1951 |
| tttgggaggc ctagacgggc ggatcacgag gtcaggagat caaaaccatc ctggctaaca                                                                         |     |     | 2011 |

|                                                                      |      |
|----------------------------------------------------------------------|------|
| cggtaaaacc ccatctctgc taaaaataca aaaattagcc gggcgtggtg gcgggcacct    | 2071 |
| gtagtcccaag ctacttggga gcctcaggcg ggagaatggt gtgaaccggg gaggcggagc   | 2131 |
| ttgcagtgtat ccgagatcggt gccactgcac tccagcctgg gcgaaagagc gagactcccc  | 2191 |
| gtctcaaaaa aaattttttt gaaaaattcg taaaccatac ttttaagatt atttcagtgg    | 2251 |
| attttaaaaa atcttgtaca gaaatcaggg ttcttagcta gcagttttc tcccacgcag     | 2311 |
| tcactgtaat gtgactatgt attgcttagat tgaataagaa aataaaataa tatcttcttc   | 2371 |
| cttgaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaagg ccggccgctc tagaggatcc | 2431 |
| ctcgaggggc ccaagct                                                   | 2448 |

|                    |
|--------------------|
| <210> 17           |
| <211> 431          |
| <212> PRT          |
| <213> Homo sapiens |
| <400> 17           |

|                                                                 |
|-----------------------------------------------------------------|
| Met Gln Ala Leu Asn Ile Thr Pro Glu Gln Phe Ser Arg Leu Leu Arg |
| 1 5 10 15                                                       |

|                                                                 |
|-----------------------------------------------------------------|
| Asp His Asn Leu Thr Arg Glu Gln Phe Ile Ala Leu Tyr Arg Leu Arg |
| 20 25 30                                                        |

|                                                                 |
|-----------------------------------------------------------------|
| Pro Leu Val Tyr Thr Pro Glu Leu Pro Gly Arg Ala Lys Leu Ala Leu |
| 35 40 45                                                        |

|                                                                 |
|-----------------------------------------------------------------|
| Val Leu Thr Gly Val Leu Ile Phe Ala Leu Ala Leu Phe Gly Asn Ala |
| 50 55 60                                                        |

|                                                                 |
|-----------------------------------------------------------------|
| Leu Val Phe Tyr Val Val Thr Arg Ser Lys Ala Met Arg Thr Val Thr |
| 65 70 75 80                                                     |

|                                                                 |
|-----------------------------------------------------------------|
| Asn Ile Phe Ile Cys Ser Leu Ala Leu Ser Asp Leu Leu Ile Thr Phe |
| 85 90 95                                                        |

|                                                                 |
|-----------------------------------------------------------------|
| Phe Cys Ile Pro Val Thr Met Leu Gln Asn Ile Ser Asp Asn Trp Leu |
| 100 105 110                                                     |

|                                                                 |
|-----------------------------------------------------------------|
| Gly Gly Ala Phe Ile Cys Lys Met Val Pro Phe Val Gln Ser Thr Ala |
| 115 120 125                                                     |

Val Val Thr Glu Ile Leu Thr Met Thr Cys Ile Ala Val Glu Arg His  
130 135 140

Gln Gly Leu Val His Pro Phe Lys Met Lys Trp Gln Tyr Thr Asn Arg  
145 150 155 160

Arg Ala Phe Thr Met Leu Gly Val Val Trp Leu Val Ala Val Ile Val  
165 170 175

Gly Ser Pro Met Trp His Val Gln Gln Leu Glu Ile Lys Tyr Asp Phe  
180 185 190

Leu Tyr Glu Lys Glu His Ile Cys Cys Leu Glu Glu Trp Thr Ser Pro  
195 200 205

Val His Gln Lys Ile Tyr Thr Phe Ile Leu Val Ile Leu Phe Leu  
210 215 220

Leu Pro Leu Met Val Met Leu Ile Leu Tyr Ser Lys Ile Gly Tyr Glu  
225 230 235 240

Leu Trp Ile Lys Lys Arg Val Gly Asp Gly Ser Val Leu Arg Thr Ile  
245 250 255

His Gly Lys Glu Met Ser Lys Ile Ala Arg Lys Lys Arg Ala Val  
260 265 270

Ile Met Met Val Thr Val Val Ala Leu Phe Ala Val Cys Trp Ala Pro  
275 280 285

Phe His Val Val His Met Met Ile Glu Tyr Ser Asn Phe Glu Lys Glu  
290 295 300

Tyr Asp Asp Val Thr Ile Lys Met Ile Phe Ala Ile Val Gln Ile Ile  
305 310 315 320

Gly Phe Ser Asn Ser Ile Cys Asn Pro Ile Val Tyr Ala Phe Met Asn  
325 330 335

Glu Asn Phe Lys Lys Asn Val Leu Ser Ala Val Cys Tyr Cys Ile Val  
340 345 350

Asn Lys Thr Phe Ser Pro Ala Gln Arg His Gly Asn Ser Gly Ile Thr

355 360 365

Met Met Arg Lys Lys Ala Lys Phe Ser Leu Arg Glu Asn Pro Val Glu  
370 375 380

Glu Thr Lys Gly Glu Ala Phe Ser Asp Gly Asn Ile Glu Val Lys Leu  
385 390 395 400

Cys Glu Gln Thr Glu Glu Lys Lys Leu Lys Arg His Leu Ala Leu  
405 410 415

Phe Arg Ser Glu Leu Ala Glu Asn Ser Pro Leu Asp Ser Gly His  
420 425 430

<210> 18  
<211> 21  
<212> DNA  
<213> Homo sapiens

<400> 18  
tttctggatc gtcagcttgc t 21

<210> 19  
<211> 21  
<212> DNA  
<213> Homo sapiens

<400> 19  
acagggtctgg tccactcttc t 21

<210> 20  
<211> 33  
<212> DNA  
<213> Homo sapiens

<400> 20  
cccaagctta tgcaggcgct taacattacc ccg 33

<210> 21  
<211> 32  
<212> DNA  
<213> Homo sapiens

<400> 21  
cgggatcctt aatgccactg tctaaaggaa ga 32

<210> 22  
<211> 68

<212> DNA  
<213> Homo sapiens

<400> 22  
cgggatcctt acttgcgtc gtcgtccttg tagtccatat gcccaactgtc taaaggagaa 60  
ttctcaac 68

<210> 23  
<211> 35  
<212> DNA  
<213> Homo sapiens

<400> 23  
gcagcagcgg ccgcatgcgc accgtcacca acatc 35

<210> 24  
<211> 37  
<212> DNA  
<213> Homo sapiens

<400> 24  
gcagcagtcg acatgccac tgtctaaagg agaattc 37

<210> 25  
<211> 39  
<212> DNA  
<213> Homo sapiens

<400> 25  
gcagcagcgg ccgcatgcag ggcgttaaca ttacccgg 39

<210> 26  
<211> 34  
<212> DNA  
<213> Homo sapiens

<400> 26  
gcagcagtcg acatattcct tttcaaaatt actg 34

<210> 27  
<211> 733  
<212> DNA  
<213> Homo sapiens

<400> 27  
gggatccgga gcccaaattct tctgacaaaa ctcacacatg cccaccgtgc ccagcacctg 60  
aattcgaggg tgcaccgtca gtcttcctct tccccccaaa acccaaggac accctcatga 120  
tctcccgac tcctgaggtc acatgcgtgg tggtgacgt aagccacgaa gaccctgagg 180

|                                                                     |     |
|---------------------------------------------------------------------|-----|
| tcaagttcaa ctgg tacgtg gacggcgtgg aggtgcataa tgccaagaca aagccgcggg  | 240 |
| aggagcagta caac agc acg taccgtgtgg tcagcgtcct caccgtcctg caccaggact | 300 |
| ggctgaatgg caaggagtaac aagt gcaagg tctccaacaa agccctccca acccccatcg | 360 |
| agaaaaccat ctccaaagcc aaaggcagc cccgagaacc acaggtgtac accctgcccc    | 420 |
| catcccggga tgagctgacc aagaaccagg tcagcctgac ctgcctggtc aaaggcttct   | 480 |
| atccaaagcga catcgccgtg gagtggaga gcaatggca gccggagaac aactacaaga    | 540 |
| ccacgcctcc cgtgctggac tccgacggct ccttcttcct ctacagcaag ctcaccgtgg   | 600 |
| acaagagcag gtggcagcag gggAACgtct tctcatgctc cgtgatgcat gaggctctgc   | 660 |
| acaaccacta cacgcagaag agcctctccc tgtctccggg taaatgagtg cgacggccgc   | 720 |
| gactctagag gat                                                      | 733 |

|                    |    |
|--------------------|----|
| <210> 28           |    |
| <211> 19           |    |
| <212> DNA          |    |
| <213> Homo sapiens |    |
| <400> 28           |    |
| ccaaccga agggctttc | 19 |

|                         |    |
|-------------------------|----|
| <210> 29                |    |
| <211> 21                |    |
| <212> DNA               |    |
| <213> Homo sapiens      |    |
| <400> 29                |    |
| ccacatgggt gatcctacga t | 21 |

|                             |    |
|-----------------------------|----|
| <210> 30                    |    |
| <211> 25                    |    |
| <212> DNA                   |    |
| <213> Homo sapiens          |    |
| <400> 30                    |    |
| actgccacca gccagaccac accta | 25 |

|                    |    |
|--------------------|----|
| <210> 31           |    |
| <211> 17           |    |
| <212> DNA          |    |
| <213> Homo sapiens |    |
| <400> 31           |    |
| catccgcctt attacat | 17 |

<210> 32  
<211> 23  
<212> DNA  
<213> Homo sapiens

<400> 32  
catccgcctt attacatctt ttt

23

<210> 33  
<211> 17  
<212> DNA  
<213> Homo sapiens

<400> 33  
catgcggggc agcgagg

17

<210> 34  
<211> 24  
<212> DNA  
<213> Homo sapiens

<400> 34  
catgcggggc agcgagggct tcgg

24

<210> 35  
<211> 15  
<212> PRT  
<213> artificial

<220>  
<223> Synthetic Peptide.

<400> 35

Gly Asp Phe Trp Tyr Glu Ala Cys Glu Ser Ser Cys Ala Phe Trp  
1 5 10 15

<210> 36  
<211> 15  
<212> PRT  
<213> artificial

<220>  
<223> Synthetic Peptide.

<400> 36

Leu Glu Trp Gly Ser Asp Val Phe Tyr Asp Val Tyr Asp Cys Cys  
1 5 10 15

<210> 37  
<211> 15  
<212> PRT  
<213> artificial

&lt;220&gt;

&lt;223&gt; Synthetic Peptide.

&lt;400&gt; 37

Cys Leu Arg Ser Gly Thr Gly Cys Ala Phe Gln Leu Tyr Arg Phe  
1 5 10 15

&lt;210&gt; 38

&lt;211&gt; 15

&lt;212&gt; PRT

&lt;213&gt; artificial

&lt;220&gt;

&lt;223&gt; Synthetic Peptide.

&lt;400&gt; 38

Phe Ala Gly Gln Ile Ile Trp Tyr Asp Ala Leu Asp Thr Leu Met  
1 5 10 15

&lt;210&gt; 39

&lt;211&gt; 397

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;400&gt; 39

Met Arg Ser Pro Ser Ala Ala Trp Leu Leu Gly Ala Ala Ile Leu Leu  
1 5 10 15

Ala Ala Ser Leu Ser Cys Ser Gly Thr Ile Gln Gly Thr Asn Arg Ser  
20 25 30

Ser Lys Gly Arg Ser Leu Ile Gly Lys Val Asp Gly Thr Ser His Val  
35 40 45

Thr Gly Lys Gly Val Thr Val Glu Thr Val Phe Ser Val Asp Glu Phe  
50 55 60

Ser Ala Ser Val Leu Thr Gly Lys Leu Thr Thr Val Phe Leu Pro Ile  
65 70 75 80

Val Tyr Thr Ile Val Phe Val Val Gly Leu Pro Ser Asn Gly Met Ala  
85 90 95

Leu Trp Val Phe Leu Phe Arg Thr Lys Lys Lys His Pro Ala Val Ile  
100 105 110

Tyr Met Ala Asn Leu Ala Asp Leu Leu Ser Val Ile Trp Phe  
115 120 125

Pro Leu Lys Ile Ala Tyr His Ile His Gly Asn Asn Trp Ile Tyr Gly  
130 135 140

Glu Ala Leu Cys Asn Val Leu Ile Gly Phe Phe Tyr Gly Asn Met Tyr

|                                                                 |     |     |     |
|-----------------------------------------------------------------|-----|-----|-----|
| 145                                                             | 150 | 155 | 160 |
| Cys Ser Ile Leu Phe Met Thr Cys Leu Ser Val Gln Arg Tyr Trp Val |     |     |     |
| 165                                                             | 170 | 175 |     |
| Ile Val Asn Pro Met Gly His Ser Arg Lys Lys Ala Asn Ile Ala Ile |     |     |     |
| 180                                                             | 185 | 190 |     |
| Gly Ile Ser Leu Ala Ile Trp Leu Leu Ile Leu Leu Val Thr Ile Pro |     |     |     |
| 195                                                             | 200 | 205 |     |
| Leu Tyr Val Val Lys Gln Thr Ile Phe Ile Pro Ala Leu Asn Ile Thr |     |     |     |
| 210                                                             | 215 | 220 |     |
| Thr Cys His Asp Val Leu Pro Glu Gln Leu Leu Val Gly Asp Met Phe |     |     |     |
| 225                                                             | 230 | 235 | 240 |
| Asn Tyr Phe Leu Ser Leu Ala Ile Gly Val Phe Leu Phe Pro Ala Phe |     |     |     |
| 245                                                             | 250 | 255 |     |
| Leu Thr Ala Ser Ala Tyr Val Leu Met Ile Arg Met Leu Arg Ser Ser |     |     |     |
| 260                                                             | 265 | 270 |     |
| Ala Met Asp Glu Asn Ser Glu Lys Lys Arg Lys Arg Ala Ile Lys Leu |     |     |     |
| 275                                                             | 280 | 285 |     |
| Ile Val Thr Val Leu Ala Met Tyr Leu Ile Cys Phe Thr Pro Ser Asn |     |     |     |
| 290                                                             | 295 | 300 |     |
| Leu Leu Leu Val Val His Tyr Phe Leu Ile Lys Ser Gln Gly Gln Ser |     |     |     |
| 305                                                             | 310 | 315 | 320 |
| His Val Tyr Ala Leu Tyr Ile Val Ala Leu Cys Leu Ser Thr Leu Asn |     |     |     |
| 325                                                             | 330 | 335 |     |
| Ser Cys Ile Asp Pro Phe Val Tyr Tyr Phe Val Ser His Asp Phe Arg |     |     |     |
| 340                                                             | 345 | 350 |     |
| Asp His Ala Lys Asn Ala Leu Leu Cys Arg Ser Val Arg Thr Val Lys |     |     |     |
| 355                                                             | 360 | 365 |     |
| Gln Met Gln Val Ser Leu Thr Ser Lys Lys His Ser Arg Lys Ser Ser |     |     |     |
| 370                                                             | 375 | 380 |     |
| Ser Tyr Ser Ser Ser Ser Thr Thr Val Lys Thr Ser Tyr             |     |     |     |
| 385                                                             | 390 | 395 |     |
| <210> 40                                                        |     |     |     |
| <211> 374                                                       |     |     |     |
| <212> PRT                                                       |     |     |     |
| <213> Homo sapiens                                              |     |     |     |
| <400> 40                                                        |     |     |     |
| Met Lys Ala Leu Ile Phe Ala Ala Ala Gly Leu Leu Leu Leu Pro     |     |     |     |
| 1                                                               | 5   | 10  | 15  |

Thr Phe Cys Gln Ser Gly Met Glu Asn Asp Thr Asn Asn Leu Ala Lys  
 20 25 30

Pro Thr Leu Pro Ile Lys Thr Phe Arg Gly Ala Pro Pro Asn Ser Phe  
 35 40 45

Glu Glu Phe Pro Phe Ser Ala Leu Glu Gly Trp Thr Gly Ala Thr Ile  
 50 55 60

Thr Val Lys Ile Lys Cys Pro Glu Glu Ser Ala Ser His Leu His Val  
 65 70 75 80

Lys Asn Ala Thr Met Gly Tyr Leu Thr Ser Ser Leu Ser Thr Lys Leu  
 85 90 95

Ile Pro Ala Ile Tyr Leu Leu Val Phe Val Val Gly Val Pro Ala Asn  
 100 105 110

Ala Val Thr Leu Trp Met Leu Phe Phe Arg Thr Arg Ser Ile Cys Thr  
 115 120 125

Thr Val Phe Tyr Thr Asn Leu Ala Ile Ala Asp Phe Leu Phe Cys Val  
 130 135 140

Thr Leu Pro Phe Lys Ile Ala Tyr His Leu Asn Gly Asn Asn Trp Val  
 145 150 155 160

Phe Gly Glu Val Leu Cys Arg Ala Thr Thr Val Ile Phe Tyr Gly Asn  
 165 170 175

Met Tyr Cys Ser Ile Leu Leu Ala Cys Ile Ser Ile Asn Arg Tyr  
 180 185 190

Leu Ala Ile Val His Pro Phe Thr Tyr Arg Gly Leu Pro Lys His Thr  
 195 200 205

Tyr Ala Leu Val Thr Cys Gly Leu Val Trp Ala Thr Val Phe Leu Tyr  
 210 215 220

Met Leu Pro Phe Phe Ile Leu Lys Gln Glu Tyr Tyr Leu Val Gln Pro  
 225 230 235 240

Asp Ile Thr Thr Cys His Asp Val His Asn Thr Cys Glu Ser Ser Ser  
 245 250 255

Pro Phe Gln Leu Tyr Tyr Phe Ile Ser Leu Ala Phe Phe Gly Phe Leu  
 260 265 270

Ile Pro Phe Val Leu Ile Ile Tyr Cys Tyr Ala Ala Ile Ile Arg Thr  
 275 280 285

Leu Asn Ala Tyr Asp His Arg Trp Leu Trp Tyr Val Lys Ala Ser Leu  
 290 295 300

Leu Ile Leu Val Ile Phe Thr Ile Cys Phe Ala Pro Ser Asn Ile Ile  
 305 310 315 320

Leu Ile Ile His His Ala Asn Tyr Tyr Tyr Asn Asn Thr Asp Gly Leu  
 325 330 335

Tyr Phe Ile Tyr Leu Ile Ala Leu Cys Leu Gly Ser Leu Asn Ser Cys  
 340 345 350

Leu Asp Pro Phe Leu Tyr Phe Leu Met Ser Lys Thr Arg Asn His Ser  
 355 360 365

Thr Ala Tyr Leu Thr Lys  
 370

<210> 41  
 <211> 420  
 <212> PRT  
 <213> Xenopus laevis

<400> 41

Met Met Glu Leu Arg Val Leu Leu Leu Leu Leu Leu Thr Leu Leu  
 1 5 10 15

Gly Ala Met Gly Ser Leu Cys Leu Ala Asn Ser Asp Thr Gln Ala Lys  
 20 25 30

Gly Ala His Ser Asn Asn Met Thr Ile Lys Thr Phe Arg Ile Phe Asp  
 35 40 45

Asp Ser Glu Ser Glu Phe Glu Glu Ile Pro Trp Asp Glu Leu Asp Glu  
 50 55 60

Ser Gly Glu Gly Ser Gly Asp Gln Ala Pro Val Ser Arg Ser Ala Arg  
 65 70 75 80

Lys Pro Ile Arg Arg Asn Ile Thr Lys Glu Ala Glu Gln Tyr Leu Ser  
 85 90 95

Ser Gln Trp Leu Thr Lys Phe Val Pro Ser Leu Tyr Thr Val Val Phe  
 100 105 110

Ile Val Gly Leu Pro Leu Asn Leu Leu Ala Ile Ile Ile Phe Leu Phe  
 115 120 125

Lys Met Lys Val Arg Lys Pro Ala Val Val Tyr Met Leu Asn Leu Ala  
 130 135 140

Ile Ala Asp Val Phe Phe Val Ser Val Leu Pro Phe Lys Ile Ala Tyr  
 145 150 155 160

His Leu Ser Gly Asn Asp Trp Leu Phe Gly Pro Gly Met Cys Arg Ile  
 165 170 175

Val Thr Ala Ile Phe Tyr Cys Asn Met Tyr Cys Ser Val Leu Leu Ile  
 180 185 190

Ala Ser Ile Ser Val Asp Arg Phe Leu Ala Val Val Tyr Pro Met His  
 195 200 205

Ser Leu Ser Trp Arg Thr Met Ser Arg Ala Tyr Met Ala Cys Ser Phe  
 210 215 220  
 Ile Trp Leu Ile Ser Ile Ala Ser Thr Ile Pro Leu Leu Val Thr Glu  
 225 230 235 240  
 Gln Thr Gln Lys Ile Pro Arg Leu Asp Ile Thr Thr Cys His Asp Val  
 245 250 255  
 Leu Asp Leu Lys Asp Leu Lys Asp Phe Tyr Ile Tyr Tyr Phe Ser Ser  
 260 265 270  
 Phe Cys Leu Leu Phe Phe Val Pro Phe Ile Ile Thr Thr Ile Cys  
 275 280 285  
 Tyr Ile Gly Ile Ile Arg Ser Leu Ser Ser Ser Ile Glu Asn Ser  
 290 295 300  
 Cys Lys Lys Thr Arg Ala Leu Phe Leu Ala Val Val Val Leu Cys Val  
 305 310 315 320  
 Phe Ile Ile Cys Phe Gly Pro Thr Asn Val Leu Phe Leu Thr His Tyr  
 325 330 335  
 Leu Gln Glu Ala Asn Glu Phe Leu Tyr Phe Ala Tyr Ile Leu Ser Ala  
 340 345 350  
 Cys Val Gly Ser Val Ser Cys Cys Leu Asp Pro Leu Ile Tyr Tyr Tyr  
 355 360 365  
 Ala Ser Ser Gln Cys Gln Arg Tyr Leu Tyr Ser Leu Leu Cys Cys Arg  
 370 375 380  
 Lys Val Ser Glu Pro Gly Ser Ser Thr Gly Gln Leu Met Ser Thr Ala  
 385 390 395 400  
 Met Lys Asn Asp Asn Cys Ser Thr Asn Ala Lys Ser Ser Ile Tyr Lys  
 405 410 415  
 Lys Leu Leu Ala  
 420  
 <210> 42  
 <211> 425  
 <212> PRT  
 <213> Homo sapiens  
 <400> 42  
 Met Gly Pro Arg Arg Leu Leu Leu Val Ala Ala Cys Phe Ser Leu Cys  
 1 5 10 15  
 Gly Pro Leu Leu Ser Ala Arg Thr Arg Ala Arg Arg Pro Glu Ser Lys  
 20 25 30  
 Ala Thr Asn Ala Thr Leu Asp Pro Arg Ser Phe Leu Leu Arg Asn Pro

| 35  | 40  | 45  |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Asn | Asp | Lys | Tyr | Glu | Pro | Phe | Trp | Glu | Asp | Glu | Glu | Lys | Asn | Glu | Ser |
| 50  |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| 55  |     |     |     |     |     |     |     |     |     |     |     |     |     |     | 60  |
| Gly | Leu | Thr | Glu | Tyr | Arg | Leu | Val | Ser | Ile | Asn | Lys | Ser | Ser | Pro | Leu |
| 65  |     |     |     |     |     |     |     |     |     |     |     |     |     |     | 80  |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| Gln | Lys | Gln | Leu | Pro | Ala | Phe | Ile | Ser | Glu | Asp | Ala | Ser | Gly | Tyr | Leu |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     | 85  |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     | 90  |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     | 95  |
| Thr | Ser | Ser | Trp | Leu | Thr | Leu | Phe | Val | Pro | Ser | Val | Tyr | Thr | Gly | Val |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     | 100 |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     | 105 |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     | 110 |
| Phe | Val | Val | Ser | Leu | Pro | Leu | Asn | Ile | Met | Ala | Ile | Val | Val | Phe | Ile |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     | 115 |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     | 120 |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     | 125 |
| Leu | Lys | Met | Lys | Val | Lys | Lys | Pro | Ala | Val | Val | Tyr | Met | Leu | His | Leu |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     | 130 |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     | 135 |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     | 140 |
| Ala | Thr | Ala | Asp | Val | Leu | Phe | Val | Ser | Val | Leu | Pro | Phe | Lys | Ile | Ser |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     | 145 |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     | 150 |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     | 155 |
| Tyr | Tyr | Phe | Ser | Gly | Ser | Asp | Trp | Gln | Phe | Gly | Ser | Glu | Leu | Cys | Arg |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     | 165 |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     | 170 |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     | 175 |
| Phe | Val | Thr | Ala | Ala | Phe | Tyr | Cys | Asn | Met | Tyr | Ala | Ser | Ile | Leu | Leu |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     | 180 |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     | 185 |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     | 190 |
| Met | Thr | Val | Ile | Ser | Ile | Asp | Arg | Phe | Leu | Ala | Val | Val | Tyr | Pro | Met |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     | 195 |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     | 200 |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     | 205 |
| Gln | Ser | Leu | Ser | Trp | Arg | Thr | Leu | Gly | Arg | Ala | Ser | Phe | Thr | Cys | Leu |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     | 210 |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     | 215 |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     | 220 |
| Ala | Ile | Trp | Ala | Leu | Ala | Ile | Ala | Gly | Val | Val | Pro | Leu | Val | Leu | Lys |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     | 225 |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     | 230 |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     | 235 |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     | 240 |
| Glu | Gln | Thr | Ile | Gln | Val | Pro | Gly | Leu | Asn | Ile | Thr | Thr | Cys | His | Asp |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     | 245 |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     | 250 |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     | 255 |
| Val | Leu | Asn | Glu | Thr | Leu | Leu | Glu | Gly | Tyr | Tyr | Ala | Tyr | Tyr | Phe | Ser |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     | 260 |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     | 265 |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     | 270 |
| Ala | Phe | Ser | Ala | Val | Phe | Phe | Phe | Val | Pro | Leu | Ile | Ile | Ser | Thr | Val |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     | 275 |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     | 280 |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     | 285 |
| Cys | Tyr | Val | Ser | Ile | Ile | Arg | Cys | Leu | Ser | Ser | Ser | Ala | Val | Ala | Asn |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     | 290 |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     | 295 |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     | 300 |
| Arg | Ser | Lys | Lys | Ser | Arg | Ala | Leu | Phe | Leu | Ser | Ala | Ala | Val | Phe | Cys |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     | 305 |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     | 310 |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     | 315 |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     | 320 |
| Ile | Phe | Ile | Ile | Cys | Phe | Gly | Pro | Thr | Asn | Val | Leu | Leu | Ile | Ala | His |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     | 325 |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     | 330 |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     | 335 |
| Tyr | Ser | Phe | Leu | Ser | His | Thr | Ser | Thr | Thr | Glu | Ala | Ala | Tyr | Phe | Ala |

|                                                                 |     |     |
|-----------------------------------------------------------------|-----|-----|
| 340                                                             | 345 | 350 |
| Tyr Leu Leu Cys Val Cys Val Ser Ser Ile Ser Ser Cys Ile Asp Pro |     |     |
| 355                                                             | 360 | 365 |
| Leu Ile Tyr Tyr Tyr Ala Ser Ser Glu Cys Gln Arg Tyr Val Tyr Ser |     |     |
| 370                                                             | 375 | 380 |
| Ile Leu Cys Cys Lys Glu Ser Ser Asp Pro Ser Ser Tyr Asn Ser Ser |     |     |
| 385                                                             | 390 | 395 |
| Gly Gln Leu Met Ala Ser Lys Met Asp Thr Cys Ser Ser Asn Leu Asn |     |     |
| 405                                                             | 410 | 415 |
| Asn Ser Ile Tyr Lys Lys Leu Leu Thr                             |     |     |
| 420                                                             | 425 |     |
| <210> 43                                                        |     |     |
| <211> 385                                                       |     |     |
| <212> PRT                                                       |     |     |
| <213> Homo sapiens                                              |     |     |
| <400> 43                                                        |     |     |
| Met Trp Gly Arg Leu Leu Leu Trp Pro Leu Val Leu Gly Phe Ser Leu |     |     |
| 1                                                               | 5   | 10  |
| 15                                                              |     |     |
| Ser Gly Gly Thr Gln Thr Pro Ser Val Tyr Asp Glu Ser Gly Ser Thr |     |     |
| 20                                                              | 25  | 30  |
| Gly Gly Gly Asp Asp Ser Thr Pro Ser Ile Leu Pro Ala Pro Arg Gly |     |     |
| 35                                                              | 40  | 45  |
| Tyr Pro Gly Gln Val Cys Ala Asn Asp Ser Asp Thr Leu Glu Leu Pro |     |     |
| 50                                                              | 55  | 60  |
| Asp Ser Ser Arg Ala Leu Leu Leu Gly Trp Val Pro Thr Arg Leu Val |     |     |
| 65                                                              | 70  | 75  |
| 80                                                              |     |     |
| Pro Ala Leu Tyr Gly Leu Val Leu Val Val Gly Leu Pro Ala Asn Gly |     |     |
| 85                                                              | 90  | 95  |
| Leu Ala Leu Trp Val Leu Ala Thr Gln Ala Pro Arg Leu Pro Ser Thr |     |     |
| 100                                                             | 105 | 110 |
| Met Leu Leu Met Asn Leu Ala Thr Ala Asp Leu Leu Leu Ala Leu Ala |     |     |
| 115                                                             | 120 | 125 |
| Leu Pro Pro Arg Ile Ala Tyr His Leu Arg Gly Gln Arg Trp Pro Phe |     |     |
| 130                                                             | 135 | 140 |
| Gly Glu Ala Ala Cys Arg Leu Ala Thr Ala Ala Leu Tyr Gly His Met |     |     |
| 145                                                             | 150 | 155 |
| 160                                                             |     |     |
| Tyr Gly Ser Val Leu Leu Leu Ala Ala Val Ser Leu Asp Arg Tyr Leu |     |     |
| 165                                                             | 170 | 175 |

Ala Leu Val His Pro Leu Arg Ala Arg Ala Leu Arg Gly Arg Arg Leu  
 180 185 190

Ala Leu Gly Leu Cys Met Ala Ala Trp Leu Met Ala Ala Ala Leu Ala  
 195 200 205

Leu Pro Leu Thr Leu Gln Arg Gln Thr Phe Arg Leu Ala Arg Ser Asp  
 210 215 220

Arg Val Leu Cys His Asp Ala Leu Pro Leu Asp Ala Gln Ala Ser His  
 225 230 235 240

Trp Gln Pro Ala Phe Thr Cys Leu Ala Leu Leu Gly Cys Phe Leu Pro  
 245 250 255

Leu Leu Ala Met Leu Leu Cys Tyr Gly Ala Thr Leu His Thr Leu Ala  
 260 265 270

Ala Ser Gly Arg Arg Tyr Gly His Ala Leu Arg Leu Thr Ala Val Val  
 275 280 285

Leu Ala Ser Ala Val Ala Phe Phe Val Pro Ser Asn Leu Leu Leu Leu  
 290 295 300

Leu His Tyr Ser Asp Pro Ser Pro Ser Ala Trp Gly Asn Leu Tyr Gly  
 305 310 315 320

Ala Tyr Val Pro Ser Leu Ala Leu Ser Thr Leu Asn Ser Cys Val Asp  
 325 330 335

Pro Phe Ile Tyr Tyr Val Ser Ala Glu Phe Arg Asp Lys Val Arg  
 340 345 350

Ala Gly Leu Phe Gln Arg Ser Pro Gly Asp Thr Val Ala Ser Lys Ala  
 355 360 365

Ser Ala Glu Gly Gly Ser Arg Gly Met Gly Thr His Ser Ser Leu Leu  
 370 375 380

Gln  
 385 ..

<210> 44  
 <211> 370  
 <212> PRT  
 <213> Homo sapiens

<400> 44

Met Gly Asp Arg Arg Phe Ile Asp Phe Gln Phe Gln Asp Ser Asn Ser  
 1 5 10 15

Ser Leu Arg Pro Arg Leu Gly Asn Ala Thr Ala Asn Asn Thr Cys Ile  
 20 25 30

Val Asp Asp Ser Phe Lys Tyr Asn Leu Asn Gly Ala Val Tyr Ser Val  
 35 40 45

Val Phe Ile Leu Gly Leu Ile Thr Asn Ser Val Ser Leu Phe Val Phe  
 50 55 60  
 Cys Phe Arg Met Lys Met Arg Ser Glu Thr Ala Ile Phe Ile Thr Asn  
 65 70 75 80  
 Leu Ala Val Ser Asp Leu Leu Phe Val Cys Thr Leu Pro Phe Lys Ile  
 85 90 95  
 Phe Tyr Asn Phe Asn Arg His Trp Pro Phe Gly Asp Thr Leu Cys Lys  
 100 105 110  
 Ile Ser Gly Thr Ala Phe Leu Thr Asn Ile Tyr Gly Ser Met Leu Phe  
 115 120 125  
 Leu Thr Cys Ile Ser Val Asp Arg Phe Leu Ala Ile Val Tyr Pro Phe  
 130 135 140  
 Arg Ser Arg Thr Ile Arg Thr Arg Arg Asn Ser Ala Ile Val Cys Ala  
 145 150 155 160  
 Gly Val Trp Ile Leu Val Leu Ser Gly Gly Ile Ser Ala Ser Leu Phe  
 165 170 175  
 Ser Thr Thr Asn Val Asn Asn Ala Thr Thr Cys Phe Glu Gly Phe  
 180 185 190  
 Ser Lys Arg Val Trp Lys Thr Tyr Leu Ser Lys Ile Thr Ile Phe Ile  
 195 200 205  
 Glu Val Val Gly Phe Ile Ile Pro Leu Ile Leu Asn Val Ser Cys Ser  
 210 215 220  
 Ser Val Val Leu Arg Thr Leu Arg Lys Pro Ala Thr Leu Ser Gln Ile  
 225 230 235 240  
 Gly Thr Asn Lys Lys Lys Val Leu Lys Met Ile Thr Val His Met Ala  
 245 250 255  
 Val Phe Val Val Cys Phe Val Pro Tyr Asn Ser Val Leu Phe Leu Tyr  
 260 265 270  
 Ala Leu Val Arg Ser Gln Ala Ile Thr Asn Cys Phe Leu Glu Arg Phe  
 275 280 285  
 Ala Lys Ile Met Tyr Pro Ile Thr Leu Cys Leu Ala Thr Leu Asn Cys  
 290 295 300  
 Cys Phe Asp Pro Phe Ile Tyr Tyr Phe Thr Leu Glu Ser Phe Gln Lys  
 305 310 315 320  
 Ser Phe Tyr Ile Asn Ala His Ile Arg Met Glu Ser Leu Phe Lys Thr  
 325 330 335  
 Glu Thr Pro Leu Thr Thr Lys Pro Ser Leu Pro Ala Ile Gln Glu Glu  
 340 345 350

Val Ser Asp Gln Thr Thr Asn Asn Gly Gly Glu Leu Met Leu Glu Ser  
355 360 365

Thr Phe  
370

<210> 45  
<211> 444  
<212> PRT  
<213> Homo sapiens

<400> 45

Met Ser Gly Thr Lys Leu Glu Asp Ser Pro Pro Cys Arg Asn Trp Ser  
1 5 10 15

Ser Ala Ser Glu Leu Asn Glu Thr Gln Glu Pro Phe Leu Asn Pro Thr  
20 25 30

Asp Tyr Asp Asp Glu Glu Phe Leu Arg Tyr Leu Trp Arg Glu Tyr Leu  
35 40 45

His Pro Lys Glu Tyr Glu Trp Val Leu Ile Ala Gly Tyr Ile Ile Val  
50 55 60

Phe Val Val Ala Leu Ile Gly Asn Val Leu Val Cys Val Ala Val Trp  
65 70 75 80

Lys Asn His His Met Arg Thr Val Thr Asn Tyr Phe Ile Val Asn Leu  
85 90 95

Ser Leu Ala Asp Val Leu Val Thr Ile Thr Cys Leu Pro Ala Thr Leu  
100 105 110

Val Val Asp Ile Thr Glu Thr Trp Phe Phe Gly Gln Ser Leu Cys Lys  
115 120 125

Val Ile Pro Tyr Leu Gln Thr Val Ser Val Ser Val Leu Thr  
130 135 140

Leu Ser Cys Ile Ala Leu Asp Arg Trp Tyr Ala Ile Cys His Pro Leu  
145 150 155 160

Met Phe Lys Ser Thr Ala Lys Arg Ala Arg Asn Ser Ile Val Ile Ile  
165 170 175

Trp Ile Val Ser Cys Ile Ile Met Ile Pro Gln Ala Ile Val Met Glu  
180 185 190

Cys Ser Thr Val Phe Pro Gly Leu Ala Asn Lys Thr Thr Leu Phe Thr  
195 200 205

Val Cys Asp Glu Arg Trp Gly Gly Glu Ile Tyr Pro Lys Met Tyr His  
210 215 220

Ile Cys Phe Phe Leu Val Thr Tyr Met Ala Pro Leu Cys Leu Met Val

|                                                                 |     |     |     |
|-----------------------------------------------------------------|-----|-----|-----|
| 225                                                             | 230 | 235 | 240 |
| Leu Ala Tyr Leu Gln Ile Phe Arg Lys Leu Trp Cys Arg Gln Ile Pro |     |     |     |
| 245                                                             | 250 | 255 |     |
| Gly Thr Ser Ser Val Val Gln Arg Lys Trp Lys Pro Leu Gln Pro Val |     |     |     |
| 260                                                             | 265 | 270 |     |
| Ser Gln Pro Arg Gly Pro Gly Gln Pro Thr Lys Ser Arg Met Ser Ala |     |     |     |
| 275                                                             | 280 | 285 |     |
| Val Ala Ala Glu Ile Lys Gln Ile Arg Ala Arg Arg Lys Thr Ala Arg |     |     |     |
| 290                                                             | 295 | 300 |     |
| Met Leu Met Val Val Leu Leu Val Phe Ala Ile Cys Tyr Leu Pro Ile |     |     |     |
| 305                                                             | 310 | 315 | 320 |
| Ser Ile Leu Asn Val Leu Lys Arg Val Phe Gly Met Phe Ala His Thr |     |     |     |
| 325                                                             | 330 | 335 |     |
| Glu Asp Arg Glu Thr Val Tyr Ala Trp Phe Thr Phe Ser His Trp Leu |     |     |     |
| 340                                                             | 345 | 350 |     |
| Val Tyr Ala Asn Ser Ala Ala Asn Pro Ile Ile Tyr Asn Phe Leu Ser |     |     |     |
| 355                                                             | 360 | 365 |     |
| Gly Lys Phe Arg Glu Glu Phe Lys Ala Ala Phe Ser Cys Cys Cys Leu |     |     |     |
| 370                                                             | 375 | 380 |     |
| Gly Val His His Arg Gln Glu Asp Arg Leu Thr Arg Gly Arg Thr Ser |     |     |     |
| 385                                                             | 390 | 395 | 400 |
| Thr Glu Ser Arg Lys Ser Leu Thr Thr Gln Ile Ser Asn Phe Asp Asn |     |     |     |
| 405                                                             | 410 | 415 |     |
| Ile Ser Lys Leu Ser Glu Gln Val Val Leu Thr Ser Ile Ser Thr Leu |     |     |     |
| 420                                                             | 425 | 430 |     |
| Pro Ala Ala Asn Gly Ala Gly Pro Leu Gln Asn Trp                 |     |     |     |
| 435                                                             | 440 |     |     |
| <210> 46                                                        |     |     |     |
| <211> 460                                                       |     |     |     |
| <212> PRT                                                       |     |     |     |
| <213> Rattus norvegicus                                         |     |     |     |
| <400> 46                                                        |     |     |     |
| Met Ser Ser Thr Lys Leu Glu Asp Ser Leu Pro Arg Arg Asn Trp Ser |     |     |     |
| 1                                                               | 5   | 10  | 15  |
| Ser Ala Ser Glu Leu Asn Glu Thr Gln Glu Pro Phe Leu Asn Pro Thr |     |     |     |
| 20                                                              | 25  | 30  |     |
| Asp Tyr Asp Asp Glu Glu Phe Leu Arg Tyr Leu Trp Arg Glu Tyr Leu |     |     |     |
| 35                                                              | 40  | 45  |     |

His Pro Lys Glu Tyr Glu Trp Val Leu Ile Ala Gly Tyr Ile Ile Val  
 50 55 60  
 Phe Val Val Ala Leu Ile Gly Asn Val Leu Val Cys Val Ala Val Trp  
 65 70 75 80  
 Lys Asn His His Met Arg Thr Val Thr Asn Tyr Phe Ile Val Asn Leu  
 85 90 95  
 Ser Leu Ala Asp Val Leu Val Thr Ile Thr Cys Leu Pro Ala Thr Leu  
 100 105 110  
 Val Val Asp Ile Thr Glu Thr Trp Phe Phe Gly Gln Ser Leu Cys Lys  
 115 120 125  
 Val Ile Pro Tyr Leu Gln Thr Val Ser Val Ser Val Val Leu Thr  
 130 135 140  
 Leu Ser Cys Ile Ala Leu Asp Arg Trp Tyr Ala Ile Cys His Pro Leu  
 145 150 155 160  
 Met Phe Lys Ser Thr Ala Lys Arg Ala Arg Asn Ser Ile Val Val Ile  
 165 170 175  
 Trp Ile Val Ser Cys Ile Ile Met Ile Pro Gln Ala Ile Val Met Glu  
 180 185 190  
 Arg Ser Ser Met Leu Pro Gly Leu Ala Asn Lys Thr Thr Leu Phe Thr  
 195 200 205  
 Val Cys Asp Glu Arg Trp Gly Gly Glu Val Tyr Pro Lys Met Tyr His  
 210 215 220  
 Ile Cys Phe Phe Leu Val Thr Tyr Met Ala Pro Leu Cys Leu Met Val  
 225 230 235 240  
 Leu Ala Tyr Leu Gln Ile Phe Arg Lys Leu Trp Cys Arg Gln Ile Pro  
 245 250 255  
 Gly Thr Ser Ser Val Val Gln Arg Lys Trp Lys Gln Pro Gln Pro Val  
 260 265 270  
 Ser Gln Pro Arg Gly Ser Gly Gln Gln Ser Lys Ala Arg Ile Ser Ala  
 275 280 285  
 Val Ala Ala Glu Ile Lys Gln Ile Arg Ala Arg Arg Lys Thr Ala Arg  
 290 295 300  
 Met Leu Met Val Val Leu Leu Val Phe Ala Ile Cys Tyr Leu Pro Ile  
 305 310 315 320  
 Ser Ile Leu Asn Val Leu Lys Arg Val Phe Gly Met Phe Thr His Thr  
 325 330 335  
 Glu Asp Arg Glu Thr Val Tyr Ala Trp Phe Thr Phe Ser His Trp Leu  
 340 345 350

Val Tyr Ala Asn Ser Ala Ala Asn Pro Ile Ile Tyr Asn Phe Leu Ser  
 355 360 365

Gly Lys Phe Arg Glu Glu Phe Lys Ala Ala Phe Ser Cys Cys Leu Gly  
 370 375 380

Val His Arg Arg Gln Gly Asp Arg Leu Ala Arg Gly Arg Thr Ser Thr  
 385 390 395 400

Glu Ser Arg Lys Ser Leu Thr Thr Gln Ile Ser Asn Phe Asp Asn Val  
 405 410 415

Ser Lys Leu Ser Glu His Val Ala Leu Thr Ser Ile Ser Thr Leu Pro  
 420 425 430

Ala Ala Asn Gly Ala Gly Pro Leu Gln Asn Trp Tyr Leu Gln Gln Gly  
 435 440 445

Val Pro Ser Ser Leu Leu Ser Thr Trp Leu Glu Val  
 450 455 460

<210> 47

<211> 375

<212> PRT

<213> Mus musculus

<400> 47

Met Asn Thr Ser His Phe Leu Ala Pro Leu Phe Pro Gly Ser Leu Gln  
 1 5 10 15

Gly Lys Asn Gly Thr Asn Pro Leu Asp Ser Pro Tyr Asn Phe Ser Asp  
 20 25 30

Gly Cys Gln Asp Ser Ala Glu Leu Leu Ala Phe Ile Ile Thr Thr Tyr  
 35 40 45

Ser Ile Glu Thr Ile Leu Gly Val Leu Gly Asn Leu Cys Leu Ile Phe  
 50 55 60

Val Thr Thr Arg Gln Lys Glu Lys Ser Asn Val Thr Asn Leu Leu Ile  
 65 70 75 80

Ala Asn Leu Ala Phe Ser Asp Phe Leu Met Cys Leu Ile Cys Gln Pro  
 85 90 95

Leu Thr Val Thr Tyr Thr Ile Met Asp Tyr Trp Ile Phe Gly Glu Val  
 100 105 110

Leu Cys Lys Met Leu Thr Phe Ile Gln Cys Met Ser Val Thr Val Ser  
 115 120 125

Ile Leu Ser Leu Val Leu Val Ala Leu Glu Arg His Gln Leu Ile Ile  
 130 135 140

Asn Pro Thr Gly Trp Lys Pro Ser Ile Phe Gln Ala Tyr Leu Gly Ile  
 145 150 155 160

Val Val Ile Trp Phe Ile Ser Cys Phe Leu Ser Leu Pro Phe Leu Ala  
 165 170 175  
 Asn Ser Thr Leu Asn Asp Leu Phe His Tyr Asn His Ser Lys Val Val  
 180 185 190  
 Glu Phe Leu Glu Asp Lys Val Val Cys Phe Val Ser Trp Ser Ser Asp  
 195 200 205  
 His His Arg Leu Ile Tyr Thr Phe Leu Leu Leu Phe Gln Tyr Cys  
 210 215 220  
 Ile Pro Leu Ala Phe Ile Leu Val Cys Tyr Ile Arg Ile Tyr Gln Arg  
 225 230 235 240  
 Leu Gln Arg Gln Lys His Val Phe His Ala His Ala Cys Ser Ser Arg  
 245 250 255  
 Ala Gly Gln Met Lys Arg Ile Asn Ser Met Leu Met Thr Met Val Thr  
 260 265 270  
 Ala Phe Ala Val Leu Trp Leu Pro Leu His Val Phe Asn Thr Leu Glu  
 275 280 285  
 Asp Trp Tyr Gln Glu Ala Ile Pro Ala Cys His Gly Asn Leu Ile Phe  
 290 295 300  
 Leu Met Cys His Leu Leu Ala Met Ala Ser Thr Cys Val Asn Pro Phe  
 305 310 315 320  
 Ile Tyr Gly Phe Leu Asn Ile Asn Phe Lys Lys Asp Ile Lys Ala Leu  
 325 330 335  
 Val Leu Thr Cys His Cys Arg Ser Pro Gln Gly Glu Ser Glu His Leu  
 340 345 350  
 Pro Leu Ser Thr Val His Thr Asp Leu Ser Lys Gly Ser Met Arg Met  
 355 360 365  
 Gly Ser Lys Ser Asn Phe Ile  
 370 375  
 <210> 48  
 <211> 375  
 <212> PRT  
 <213> Rattus norvegicus  
 <400> 48  
 Met Asn Thr Ser His Leu Met Ala Ser Leu Ser Pro Ala Phe Leu Gln  
 1 5 10 15  
 Gly Lys Asn Gly Thr Asn Pro Leu Asp Ser Leu Tyr Asn Leu Ser Asp  
 20 25 30  
 Gly Cys Gln Asp Ser Ala Asp Leu Leu Ala Phe Ile Ile Thr Thr Tyr

| 35                                                              | 40  | 45  |
|-----------------------------------------------------------------|-----|-----|
| Ser Val Glu Thr Val Leu Gly Val Leu Gly Asn Leu Cys Leu Ile Phe |     |     |
| 50                                                              | 55  | 60  |
| Val Thr Thr Arg Gln Lys Glu Lys Ser Asn Val Thr Asn Leu Leu Ile |     |     |
| 65                                                              | 70  | 75  |
| 80                                                              |     |     |
| Ala Asn Leu Ala Phe Ser Asp Phe Leu Met Cys Leu Ile Cys Gln Pro |     |     |
| 85                                                              | 90  | 95  |
| Leu Thr Val Thr Tyr Thr Ile Met Asp Tyr Trp Ile Phe Gly Glu Val |     |     |
| 100                                                             | 105 | 110 |
| Leu Cys Lys Met Leu Thr Phe Ile Gln Cys Met Ser Val Thr Val Ser |     |     |
| 115                                                             | 120 | 125 |
| Ile Leu Ser Leu Val Leu Val Ala Leu Glu Arg His Gln Leu Ile Ile |     |     |
| 130                                                             | 135 | 140 |
| Asn Pro Thr Gly Trp Lys Pro Ser Ile Ser Gln Ala Tyr Leu Gly Ile |     |     |
| 145                                                             | 150 | 155 |
| 160                                                             |     |     |
| Val Val Ile Trp Phe Ile Ser Cys Phe Leu Ser Leu Pro Phe Leu Ala |     |     |
| 165                                                             | 170 | 175 |
| Asn Ser Ile Leu Asn Asp Leu Phe His Tyr Asn His Ser Lys Val Val |     |     |
| 180                                                             | 185 | 190 |
| Glu Phe Leu Glu Asp Lys Val Val Cys Phe Val Ser Trp Ser Ser Asp |     |     |
| 195                                                             | 200 | 205 |
| His His Arg Leu Ile Tyr Thr Thr Phe Leu Leu Leu Phe Gln Tyr Cys |     |     |
| 210                                                             | 215 | 220 |
| Val Pro Leu Ala Phe Ile Leu Val Cys Tyr Met Arg Ile Tyr Gln Arg |     |     |
| 225                                                             | 230 | 235 |
| 240                                                             |     |     |
| Leu Gln Arg Gln Arg Arg Ala Phe His Thr His Thr Cys Ser Ser Arg |     |     |
| 245                                                             | 250 | 255 |
| Val Gly Gln Met Lys Arg Ile Asn Gly Met Leu Met Ala Met Val Thr |     |     |
| 260                                                             | 265 | 270 |
| Ala Phe Ala Val Leu Trp Leu Pro Leu His Val Phe Asn Thr Leu Glu |     |     |
| 275                                                             | 280 | 285 |
| Asp Trp Tyr Gln Glu Ala Ile Pro Ala Cys His Gly Asn Leu Ile Phe |     |     |
| 290                                                             | 295 | 300 |
| Leu Met Cys His Leu Phe Ala Met Ala Ser Thr Cys Val Asn Pro Phe |     |     |
| 305                                                             | 310 | 315 |
| 320                                                             |     |     |
| Ile Tyr Gly Phe Leu Asn Ile Asn Phe Lys Lys Asp Ile Lys Ala Leu |     |     |
| 325                                                             | 330 | 335 |
| Val Leu Thr Cys Arg Cys Arg Pro Pro Gln Gly Glu Pro Glu Pro Leu |     |     |

|                                                                 |     |     |
|-----------------------------------------------------------------|-----|-----|
| 340                                                             | 345 | 350 |
| Pro Leu Ser Thr Val His Thr Asp Leu Ser Lys Gly Ser Met Arg Met |     |     |
| 355                                                             | 360 | 365 |
| Gly Ser Lys Ser Asn Val Met                                     |     |     |
| 370                                                             | 375 |     |
| <210> 49                                                        |     |     |
| <211> 371                                                       |     |     |
| <212> PRT                                                       |     |     |
| <213> Oryctolagus cuniculus                                     |     |     |
| <400> 49                                                        |     |     |
| Met Glu Val Ser Leu Asn Asp Pro Ala Ser Asn Lys Thr Ser Ala Lys |     |     |
| 1                                                               | 5   | 10  |
| 15                                                              |     |     |
| Ser Asn Ser Ser Ala Phe Phe Tyr Phe Glu Ser Cys Gln Ser Pro Ser |     |     |
| 20                                                              | 25  | 30  |
| Leu Ala Leu Leu Leu Leu Ile Ala Tyr Thr Val Val Leu Ile Met     |     |     |
| 35                                                              | 40  | 45  |
| Gly Ile Cys Gly Asn Leu Ser Leu Ile Thr Ile Ile Phe Lys Lys Gln |     |     |
| 50                                                              | 55  | 60  |
| Arg Glu Ala Gln Asn Val Thr Asn Ile Leu Ile Ala Asn Leu Ser Leu |     |     |
| 65                                                              | 70  | 75  |
| 80                                                              |     |     |
| Ser Asp Ile Leu Val Cys Val Met Cys Ile Pro Phe Thr Ala Ile Tyr |     |     |
| 85                                                              | 90  | 95  |
| Thr Leu Met Asp Arg Trp Ile Phe Gly Asn Thr Met Cys Lys Leu Thr |     |     |
| 100                                                             | 105 | 110 |
| Ser Tyr Val Gln Ser Val Ser Ile Ser Val Ser Ile Phe Ser Leu Val |     |     |
| 115                                                             | 120 | 125 |
| Leu Ile Ala Ile Glu Arg Tyr Gln Leu Ile Val Asn Pro Arg Gly Trp |     |     |
| 130                                                             | 135 | 140 |
| Lys Pro Ser Ala Ser His Ala Tyr Trp Gly Ile Met Leu Ile Trp Leu |     |     |
| 145                                                             | 150 | 155 |
| 160                                                             |     |     |
| Phe Ser Leu Leu Ser Ile Pro Leu Leu Leu Ser Tyr His Leu Thr     |     |     |
| 165                                                             | 170 | 175 |
| Asp Glu Pro Phe Arg Asn Leu Ser Leu Pro Thr Asp Leu Tyr Ser His |     |     |
| 180                                                             | 185 | 190 |
| His Val Val Cys Val Glu His Trp Pro Ser Lys Thr Asn Gln Leu Leu |     |     |
| 195                                                             | 200 | 205 |
| Tyr Ser Thr Ser Leu Ile Met Leu Gln Tyr Phe Val Pro Leu Gly Phe |     |     |
| 210                                                             | 215 | 220 |

Met Phe Ile Cys Tyr Leu Lys Ile Val Ile Cys Leu His Lys Arg Asn  
 225 230 235 240

Ser Lys Ile Asp Arg Arg Arg Glu Asn Glu Ser Arg Leu Thr Glu Asn  
 245 250 255

Lys Arg Ile Asn Thr Met Leu Ile Ser Ile Val Val Thr Phe Ala Ala  
 260 265 270

Cys Trp Leu Pro Leu Asn Thr Phe Asn Val Ile Phe Asp Trp Tyr His  
 275 280 285

Glu Val Leu Met Ser Cys His His Asp Leu Val Phe Ala Ile Cys His  
 290 295 300

Leu Val Ala Met Val Ser Thr Cys Ile Asn Pro Leu Phe Tyr Gly Phe  
 305 310 315 320

Leu Asn Arg Asn Phe Gln Lys Asp Leu Val Val Leu Ile His His Cys  
 325 330 335

Leu Cys Phe Ala Leu Arg Glu Arg Tyr Glu Asn Ile Ala Ile Ser Thr  
 340 345 350

Leu His Thr Asp Glu Ser Lys Gly Ser Leu Arg Val Ala His Ile Pro  
 355 360 365

Ala Gly Ile  
 370

<210> 50  
 <211> 383  
 <212> PRT  
 <213> Cavia porcellus

<400> 50

Met Asn Ser Thr Ser Phe Ser Gln Leu Glu Asn His Ser Val His Tyr  
 1 5 10 15

Asn Leu Ser Glu Glu Lys Pro Ser Phe Phe Ala Phe Glu Asn Asp Asp  
 20 25 30

Cys His Leu Pro Leu Ala Val Ile Phe Thr Leu Ala Leu Ala Tyr Gly  
 35 40 45

Ala Val Ile Ile Leu Gly Val Ser Gly Asn Leu Ala Leu Ile Leu Ile  
 50 55 60

Ile Leu Lys Gln Lys Glu Met Arg Asn Val Thr Asn Ile Leu Ile Val  
 65 70 75 80

Asn Leu Ser Phe Ser Asp Leu Leu Val Ala Ile Met Cys Leu Pro Phe  
 85 90 95

Thr Phe Val Tyr Thr Leu Met Asp His Trp Ile Phe Gly Glu Ile Met  
 100 105 110

Cys Lys Leu Asn Pro Phe Val Gln Cys Val Ser Ile Thr Val Ser Ile  
 115 120 125

Phe Ser Leu Val Leu Ile Ala Val Glu Arg His Gln Leu Ile Ile Asn  
 130 135 140

Pro Arg Gly Trp Arg Pro Asn Asn Arg His Ala Tyr Ile Gly Ile Ala  
 145 150 155 160

Val Ile Trp Val Leu Ala Val Ala Ser Ser Leu Pro Phe Met Ile Tyr  
 165 170 175

Gln Val Leu Thr Asp Glu Pro Phe Gln Asn Val Thr Leu Asp Ala Phe  
 180 185 190

Lys Asp Lys Leu Val Cys Phe Asp Gln Phe Pro Ser Asp Ser His Arg  
 195 200 205

Leu Ser Tyr Thr Thr Leu Leu Val Leu Gln Tyr Phe Gly Pro Leu  
 210 215 220

Cys Phe Ile Phe Ile Cys Tyr Phe Lys Ile Tyr Ile Arg Leu Lys Arg  
 225 230 235 240

Arg Asn Asn Met Met Asp Lys Met Arg Asp Ser Lys Tyr Arg Ser Ser  
 245 250 255

Glu Ser Lys Arg Ile Asn Ile Met Leu Leu Ser Ile Val Val Ala Phe  
 260 265 270

Ala Val Cys Trp Leu Pro Leu Thr Ile Phe Asn Thr Val Phe Asp Trp  
 275 280 285

Asn His Gln Ile Ile Ala Thr Cys Asn His Asn Leu Leu Phe Leu Leu  
 290 295 300

Cys His Leu Thr Ala Met Ile Ser Thr Cys Val Asn Pro Ile Phe Tyr  
 305 310 315 320

Gly Phe Leu Asn Lys Asn Phe Gln Arg Asp Leu Gln Phe Phe Asn  
 325 330 335

Phe Cys Asp Phe Arg Ser Arg Asp Asp Tyr Glu Thr Ile Ala Met  
 340 345 350

Ser Thr Met His Thr Asp Val Ser Lys Thr Ser Leu Lys Gln Ala Ser  
 355 360 365

Pro Leu Ala Phe Lys Lys Ile Ser Cys Val Glu Asn Glu Lys Ile  
 370 375 380

<210> 51  
 <211> 375  
 <212> PRT  
 <213> Danio rerio

&lt;400&gt; 51

Met Glu Arg Ser His Leu Asn Asn Ser Ser Trp Leu Leu Glu Asp Pro  
 1 5 10 15

Thr Cys Pro Ala Ser Leu Ser Ser Thr Thr Phe Leu Ile Val Ala Tyr  
 20 25 30

Ser Thr Met Leu Ala Val Gly Leu Val Gly Asn Thr Cys Leu Val Val  
 35 40 45

Val Ile Thr Arg Gln Lys Glu Met Arg Asn Val Thr Asn Ile Phe Ile  
 50 55 60

Val Asn Leu Ser Cys Ser Asp Ile Leu Val Cys Leu Val Cys Leu Pro  
 65 70 75 80

Val Thr Ile Ile Tyr Thr Leu Met Asp Arg Trp Ile Leu Gly Glu Ala  
 85 90 95

Leu Cys Lys Val Thr Pro Phe Val Gln Cys Met Ser Val Thr Val Ser  
 100 105 110

Ile Phe Ser Met Val Leu Ile Ala Leu Glu Arg His Gln Leu Ile Ile  
 115 120 125

His Pro Thr Gly Trp Lys Pro Val Val Arg His Ser Tyr Leu Ala Val  
 130 135 140

Ala Val Ile Trp Ile Ile Ala Cys Phe Ile Ser Leu Pro Phe Leu Ser  
 145 150 155 160

Phe Asn Ile Leu Thr Asn Ser Pro Phe His Asn Leu Ser Leu Pro Phe  
 165 170 175

Asn Pro Phe Ser Asp His Phe Ile Cys Ile Glu Gln Trp Pro Ser Glu  
 180 185 190

Gly Asn Arg Leu Thr Tyr Thr Thr Leu Leu Leu Cys Gln Tyr Cys  
 195 200 205

Leu Pro Leu Ala Leu Ile Leu Val Cys Tyr Phe Arg Ile Phe Leu Arg  
 210 215 220

Leu Ser Arg Arg Lys Asp Met Val Glu Arg Ala Arg Gly Gly Arg Gln  
 225 230 235 240

Lys Lys Ala Lys Gly Ser Lys Arg Val Asn Ala Met Leu Ala Ser Ile  
 245 250 255

Val Ala Ala Phe Ala Leu Cys Trp Leu Pro Leu Asn Val Phe Asn Thr  
 260 265 270

Ile Phe Asp Trp Asn His Glu Ala Ile Pro Val Cys Gln His Asp Ala  
 275 280 285

Ile Phe Ser Ala Cys His Leu Thr Ala Met Ala Ser Thr Cys Val Asn

290

295

300

Pro Val Ile Tyr Gly Phe Leu Asn Asn Asn Phe Gln Lys Glu Leu Lys  
 305 310 315 320

Ser Leu Leu Ser Arg Cys Arg Cys Trp Gly Pro Ala Glu Ser Tyr Glu  
 325 330 335

Ser Phe Pro Leu Ser Thr Val Ser Thr Gly Ile Thr Lys Gly Ser Ile  
 340 345 350

Leu Ser Asn Gly Ser Ala Ser Thr Tyr Gln Pro His Lys Lys Asn Ser  
 355 360 365

Leu Glu Gln Lys Glu Ser Ile  
 370 375

<210> 52

<211> 381

<212> PRT

<213> Homo sapiens

<400> 52

Met Gly Pro Ile Gly Ala Glu Ala Asp Glu Asn Gln Thr Val Glu Glu  
 1 5 10 15

Met Lys Val Glu Gln Tyr Gly Pro Gln Thr Thr Pro Arg Gly Glu Leu  
 20 25 30

Val Pro Asp Pro Glu Pro Glu Leu Ile Asp Ser Thr Lys Leu Ile Glu  
 35 40 45

Val Gln Val Val Leu Ile Leu Ala Tyr Cys Ser Ile Ile Leu Leu Gly  
 50 55 60

Val Ile Gly Asn Ser Leu Val Ile His Val Val Ile Lys Phe Lys Ser  
 65 70 75 80

Met Arg Thr Val Thr Asn Phe Phe Ile Ala Asn Leu Ala Val Ala Asp  
 85 90 95

Leu Leu Val Asn Thr Leu Cys Leu Pro Phe Thr Leu Thr Tyr Thr Leu  
 100 105 110

Met Gly Glu Trp Lys Met Gly Pro Val Leu Cys His Leu Val Pro Tyr  
 115 120 125

Ala Gln Gly Leu Ala Val Gln Val Ser Thr Ile Thr Leu Thr Val Ile  
 130 135 140

Ala Leu Asp Arg His Arg Cys Ile Val Tyr His Leu Glu Ser Lys Ile  
 145 150 155 160

Ser Lys Arg Ile Ser Phe Leu Ile Ile Gly Leu Ala Trp Gly Ile Ser  
 165 170 175

Ala Leu Leu Ala Ser Pro Leu Ala Ile Phe Arg Glu Tyr Ser Leu Ile  
 180 185 190

Glu Ile Ile Pro Asp Phe Glu Ile Val Ala Cys Thr Glu Lys Trp Pro  
 195 200 205

Gly Glu Glu Lys Ser Ile Tyr Gly Thr Val Tyr Ser Leu Ser Ser Leu  
 210 215 220

Leu Ile Leu Tyr Val Leu Pro Leu Gly Ile Ile Ser Phe Ser Tyr Thr  
 225 230 235 240

Arg Ile Trp Ser Lys Leu Lys Asn His Val Ser Pro Gly Ala Ala Asn  
 245 250 255

Asp His Tyr His Gln Arg Arg Gln Lys Thr Thr Lys Met Leu Val Cys  
 260 265 270

Val Val Val Val Phe Ala Val Ser Trp Leu Pro Leu His Ala Phe Gln  
 275 280 285

Leu Ala Val Asp Ile Asp Ser Gln Val Leu Asp Leu Lys Glu Tyr Lys  
 290 295 300

Leu Ile Phe Thr Val Phe His Ile Ile Ala Met Cys Ser Thr Phe Ala  
 305 310 315 320

Asn Pro Leu Leu Tyr Gly Trp Met Asn Ser Asn Tyr Arg Lys Ala Phe  
 325 330 335

Leu Ser Ala Phe Arg Cys Glu Gln Arg Leu Asp Ala Ile His Ser Glu  
 340 345 350

Val Ser Val Thr Phe Lys Ala Lys Lys Asn Leu Glu Val Arg Lys Asn  
 355 360 365

Ser Gly Pro Asn Asp Ser Phe Thr Glu Ala Thr Asn Val  
 370 375 380

<210> 53

<211> 522

<212> PRT

<213> Homo sapiens

<400> 53

Met Asn Ser Phe Phe Gly Thr Pro Ala Ala Ser Trp Cys Leu Leu Glu  
 1 5 10 15

Ser Asp Val Ser Ser Ala Pro Asp Lys Glu Ala Gly Arg Glu Arg Arg  
 20 25 30

Ala Leu Ser Val Gln Gln Arg Gly Gly Pro Ala Trp Ser Gly Ser Leu  
 35 40 45

Glu Trp Ser Arg Gln Ser Ala Gly Asp Arg Arg Arg Leu Gly Leu Ser  
 50 55 60

Arg Gln Thr Ala Lys Ser Ser Trp Ser Arg Ser Arg Asp Arg Thr Cys  
 65 70 75 80  
 Cys Cys Arg Arg Ala Trp Trp Ile Leu Val Pro Ala Ala Asp Arg Ala  
 85 90 95  
 Arg Arg Glu Arg Phe Ile Met Asn Glu Lys Trp Asp Thr Asn Ser Ser  
 100 105 110  
 Glu Asn Trp His Pro Ile Trp Asn Val Asn Asp Thr Lys His His Leu  
 115 120 125  
 Tyr Ser Asp Ile Asn Ile Thr Tyr Val Asn Tyr Tyr Leu His Gln Pro  
 130 135 140  
 Gln Val Ala Ala Ile Phe Ile Ile Ser Tyr Phe Leu Ile Phe Phe Leu  
 145 150 155 160  
 Cys Met Met Gly Asn Thr Val Val Cys Phe Ile Val Met Arg Asn Lys  
 165 170 175  
 His Met His Thr Val Thr Asn Leu Phe Ile Leu Asn Leu Ala Ile Ser  
 180 185 190  
 Asp Leu Leu Val Gly Ile Phe Cys Met Pro Ile Thr Leu Leu Asp Asn  
 195 200 205  
 Ile Ile Ala Gly Trp Pro Phe Gly Asn Thr Met Cys Lys Ile Ser Gly  
 210 215 220  
 Leu Val Gln Gly Ile Ser Val Ala Ala Ser Val Phe Thr Leu Val Ala  
 225 230 235 240  
 Ile Ala Val Asp Arg Phe Gln Cys Val Val Tyr Pro Phe Lys Pro Lys  
 245 250 255  
 Leu Thr Ile Lys Thr Ala Phe Val Ile Ile Met Ile Ile Trp Val Leu  
 260 265 270  
 Ala Ile Thr Ile Met Ser Pro Ser Ala Val Met Leu His Val Gln Glu  
 275 280 285  
 Glu Lys Tyr Tyr Arg Val Arg Leu Asn Ser Gln Asn Lys Thr Ser Pro  
 290 295 300  
 Val Tyr Trp Cys Arg Glu Asp Trp Pro Asn Gln Glu Met Arg Lys Ile  
 305 310 315 320  
 Tyr Thr Thr Val Leu Phe Ala Asn Ile Tyr Leu Ala Pro Leu Ser Leu  
 325 330 335  
 Ile Val Ile Met Tyr Gly Arg Ile Gly Ile Ser Leu Phe Arg Ala Ala  
 340 345 350  
 Val Pro His Thr Gly Arg Lys Asn Gln Glu Gln Trp His Val Val Ser  
 355 360 365

Arg Lys Lys Gln Lys Ile Ile Lys Met Leu Leu Ile Val Ala Leu Leu  
 370 375 380

Phe Ile Leu Ser Trp Leu Pro Leu Trp Thr Leu Met Met Leu Ser Asp  
 385 390 395 400

Tyr Ala Asp Leu Ser Pro Asn Glu Leu Gln Ile Ile Asn Ile Tyr Ile  
 405 410 415

Tyr Pro Phe Ala His Trp Leu Ala Phe Gly Asn Ser Ser Val Asn Pro  
 420 425 430

Ile Ile Tyr Gly Phe Phe Asn Glu Asn Phe Arg Arg Gly Phe Gln Glu  
 435 440 445

Ala Phe Gln Leu Gln Leu Cys Gln Lys Arg Ala Lys Pro Met Glu Ala  
 450 455 460

Tyr Thr Leu Lys Ala Lys Ser His Val Leu Ile Asn Thr Ser Asn Gln  
 465 470 475 480

Leu Val Gln Glu Ser Thr Phe Gln Asn Pro His Gly Glu Thr Leu Leu  
 485 490 495

Tyr Arg Lys Ser Ala Glu Lys Pro Gln Gln Glu Leu Val Met Glu Glu  
 500 505 510

Leu Lys Glu Thr Thr Asn Ser Ser Glu Ile  
 515 520

<210> 54  
 <211> 348  
 <212> PRT  
 <213> Mus musculus

<400> 54

Met Glu Leu Ala Met Val Asn Leu Ser Glu Gly Asn Gly Ser Asp Pro  
 1 5 10 15

Glu Pro Pro Ala Pro Glu Ser Arg Pro Leu Phe Gly Ile Gly Val Glu  
 20 25 30

Asn Phe Ile Thr Leu Val Val Phe Gly Leu Ile Phe Ala Met Gly Val  
 35 40 45

Leu Gly Asn Ser Leu Val Ile Thr Val Leu Ala Arg Ser Lys Pro Gly  
 50 55 60

Lys Pro Arg Ser Thr Thr Asn Leu Phe Ile Leu Asn Leu Ser Ile Ala  
 65 70 75 80

Asp Leu Ala Tyr Leu Leu Phe Cys Ile Pro Phe Gln Ala Thr Val Tyr  
 85 90 95

Ala Leu Pro Thr Trp Val Leu Gly Ala Phe Ile Cys Lys Phe Ile His

| 100                                                             | 105 | 110 |     |
|-----------------------------------------------------------------|-----|-----|-----|
| Tyr Phe Phe Thr Val Ser Met Leu Val Ser Ile Phe Thr Leu Ala Ala |     |     |     |
| 115                                                             | 120 | 125 |     |
| Met Ser Val Asp Arg Tyr Val Ala Ile Val His Ser Arg Arg Ser Ser |     |     |     |
| 130                                                             | 135 | 140 |     |
| Ser Leu Arg Val Ser Arg Asn Ala Leu Leu Gly Val Gly Phe Ile Trp |     |     |     |
| 145                                                             | 150 | 155 | 160 |
| Ala Leu Ser Ile Ala Met Ala Ser Pro Val Ala Tyr His Gln Arg Leu |     |     |     |
| 165                                                             | 170 | 175 |     |
| Phe His Arg Asp Ser Asn Gln Thr Phe Cys Trp Glu Gln Trp Pro Asn |     |     |     |
| 180                                                             | 185 | 190 |     |
| Lys Leu His Lys Lys Ala Tyr Val Val Cys Thr Phe Val Phe Gly Tyr |     |     |     |
| 195                                                             | 200 | 205 |     |
| Leu Leu Pro Leu Leu Leu Ile Cys Phe Cys Tyr Ala Lys Val Leu Asn |     |     |     |
| 210                                                             | 215 | 220 |     |
| His Leu His Lys Lys Leu Lys Asn Met Ser Lys Lys Ser Glu Ala Ser |     |     |     |
| 225                                                             | 230 | 235 | 240 |
| Lys Lys Lys Thr Ala Gln Thr Val Leu Val Val Val Val Phe Gly     |     |     |     |
| 245                                                             | 250 | 255 |     |
| Ile Ser Trp Leu Pro His His Val Val His Leu Trp Ala Glu Phe Gly |     |     |     |
| 260                                                             | 265 | 270 |     |
| Ala Phe Pro Leu Thr Pro Ala Ser Phe Phe Phe Arg Ile Thr Ala His |     |     |     |
| 275                                                             | 280 | 285 |     |
| Cys Leu Ala Tyr Ser Asn Ser Ser Val Asn Pro Ile Ile Tyr Ala Phe |     |     |     |
| 290                                                             | 295 | 300 |     |
| Leu Ser Glu Asn Phe Arg Lys Ala Tyr Lys Gln Val Phe Lys Cys His |     |     |     |
| 305                                                             | 310 | 315 | 320 |
| Val Cys Asp Glu Ser Pro Arg Ser Glu Thr Lys Glu Asn Lys Ser Arg |     |     |     |
| 325                                                             | 330 | 335 |     |
| Met Asp Thr Pro Pro Ser Thr Asn Cys Thr His Val                 |     |     |     |
| 340                                                             | 345 |     |     |
| <210> 55                                                        |     |     |     |
| <211> 38                                                        |     |     |     |
| <212> DNA                                                       |     |     |     |
| <213> Homo sapiens                                              |     |     |     |
| <400> 55                                                        |     |     |     |
| gcagcagcgg ccgccggcgc atggggccca gatccccg                       |     | 38  |     |
| <210> 56                                                        |     |     |     |

<211> 37  
<212> DNA  
<213> Homo sapiens

<400> 56  
gcagcagtcg acgaacacac tctcctgcct ctggagg

37

<210> 57  
<211> 39  
<212> DNA  
<213> Homo sapiens

<400> 57  
gcagcagcgg ccgcattgcag gtcccgaaaca gcaccggcc

39

<210> 58  
<211> 37  
<212> DNA  
<213> Homo sapiens

<400> 58  
gcagcagtcg accttgtaca cgtggtagta gctcttg

37